Original Article

Risk factors for Shiga toxin-producing *Escherichia coli* infections in preadolescent schoolchildren in Buenos Aires, Argentina

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Abstract

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) infections are the leading cause of hemolytic uremic syndrome (HUS). STEC is the most common cause of acute kidney disease, responsible for 20% of renal transplants in Argentina.

Methodology: In 2007, an epidemiological survey was conducted among 883 students from the fifth and sixth years of elementary education in the public schools of San Martin City, Buenos Aires, Argentina. Degree of exposure to the known risk factors previously detected in the region as primary causes of STEC infections was evaluated. Risk factors assessed included consumption of hamburgers, poor personal hygiene, and exposure to various types of drinking and recreational water. The study was designed to evaluate exposure to risk factors for STEC infections among different socioeconomic groups.

Results: Ninety-five percent of children surveyed reported consumption of hamburgers. Most of these hamburgers were precooked. Children of high and medium strata attended private swimming-pools, while children from the low stratum attended public pools. Only 30.2% of students washed their hands after going to the toilet and only 43.5% reported hand-washing before eating.

Conclusions: Students demonstrated high levels of exposure to identified risk factors for STEC infections. Reduction of these risks will require cultural changes aimed at decreasing morbidity caused by food-borne infections. Institutional framework must provide the necessary resources to implement these changes and emphasize the importance of good personal hygiene. Health education must be implemented to increase food safety awareness of the consumers.

Key words: Shiga toxin-producing *Escherichia coli*; risk factors; food-borne disease

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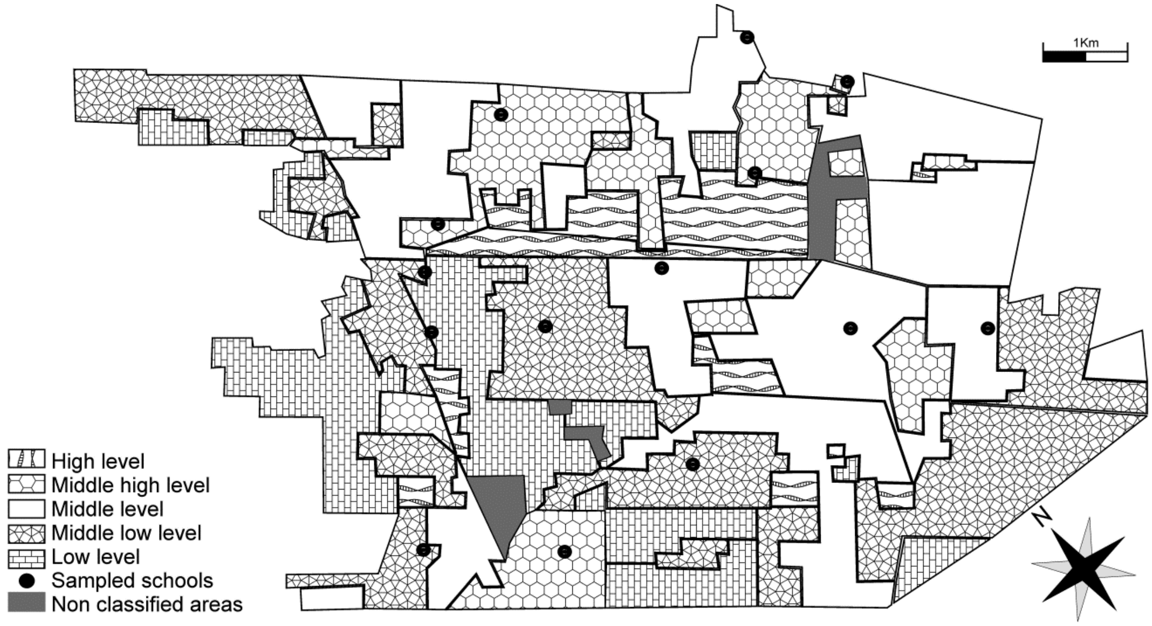
Introduction

Shiga toxing-producing *Escherichia coli* (STEC) is one of the most important groups of emerging food-borne pathogens of worldwide distribution and the principal cause of hemolytic uremic syndrome (HUS). This syndrome, characterized by acute renal failure, hemolytic anemia and thrombocytopenia, is usually secondary to acute infectious diarrhoea [1]. Most cases of HUS are identified in children younger than 16 years old with a high incidence in children up to five years of age [2-6]. In Argentina, HUS is endemic with over 450 sporadic cases recorded per year. HUS is directly responsible for 20% of pediatric renal transplants in Argentina, and represents a critical area for further research [7-8]. Almost all diarrhoea-associated HUS cases in children are due to STEC infections belonging to O157:H7 or non O157:H7 serotypes [9]. The normal habitat of STEC is the intestinal tract of ruminants and other animals; particularly sheep and cattle serve as reservoirs [10]. The prevalence of STEC in ruminants is reported to be 66.6% in sheep, 56.1% in goats, and 21.1% in cattle [11]. The main route of human exposure is fecal-oral, usually due to fecal contamination of foods. In humans, less than 100 bacteria per gram of food are necessary to establish STEC infection [12]. The World Health Organization (WHO) has classified illness from STEC as a food-borne disease (FD) [13].

Transmission of disease can also occur through direct contact of humans with carrier animals, or

Figure 1. Distribution of schools surveyed in various socioeconomic levels of San Martin, Buenos Aires, Argentina



through person-to-person transmission. Food is identified as the source of infection in approximately half of all reported cases [14]. Previous studies have evaluated the importance of various risk factors for infection in humans. Contamination of ground meat can occur during processing or preparation and represents a potentially significant source of STEC. Bacteria are destroyed at 72°C, but it is necessary to ensure that this temperature penetrates the whole of the meat prior to consumption [15]. Epidemiological studies have identified a number of useful measures to prevent possible human exposure to food-borne STEC infections. These include thorough cooking of meat (particularly ground meat), avoidance of cross-contamination during food preparation, access to safe drinking water, consumption of pasteurized milk and juices, as well as public education on the importance of good personal hygiene and cleanliness of eating utensils [16,17]. These measures complement actions aimed at lowering the prevalence of STEC in cattle [18], observing good hygiene practices during slaughter in meat processing plants, and careful preparation of meat for distribution to consumers [19].

The relationship between exposure to STEC and associated morbidity from diarrhoea among different socioeconomic groups has been extensively demonstrated [20]. Unsafe water, poor sanitation, and poor personal hygiene are three of the 10 identified risk factors for exposure to STEC. Worldwide, approximately 1.7 million deaths per year from infectious diarrhoea are attributable to these three factors [21]. Recent epidemiological results highlight the importance of socioeconomic risk factors in rates of disease associated with *E. coli* O157 [22]. Nevertheless, how socioeconomic level relates to these individual risk factors is unknown and warrants further evaluation [23].

The objective of the current study was to evaluate degree of exposure to STEC in the most vulnerable group: young schoolchildren. This goal was accomplished by assessing exposure to known risk factors for the development of HUS and their relation to the socioeconomic level of schoolchildren from urban areas in Buenos Aires.

Methodology

Study group

In total, 883 questionnaire surveys targeted at children in the 5th and 6th years of primary school (ages 10 to 12 years) were sent by professional survey-takers. The children attended 14 of the 56 state schools in an urban area of Buenos Aires, San Martin district, during 2007. This district is representative of the urban ring of Buenos Aires. Ethical approval for the study was obtained from the Department of Promotion and Prevention of Public Health Services of San Martin district.

The study was stratified by socioeconomic level because of the known association between the prevalence of childhood diarrhoea and economic inequalities. Documentary information was obtained from the National Institute of Statistics and Census

(INDEC), which defines the socioeconomic levels of the district of San Martin as high, medium-high, medium, medium-low and low [24] (Figure 1).

The 56 tuition-free public schools of the district were geo-referenced and included in the distribution maps of socioeconomic level through a system of Cartesian coordinate axes.

For this study, the five socioeconomic levels (INDEC) were partially combined to create three groups: high (high and medium-high), medium (medium) and low (medium-low and low). Students representing 25% of each socioeconomic level were selected by simple random sampling from each school.

Survey

Grade, school attended, and area were recorded for each student. Exposure to known multiple factors was assessed. The following factors were included in the assessment:

1. Consumption of hamburgers
    a. meat purchased from supermarkets
    b. commercially butchered meat
    c. home prepared hamburgers
    d. meat purchased precooked for consumption
2. Type of water available for consumption
    a. public drinking water from wells
    b. commercially bottled water
    c. other types of drinks frequently consumed
3. Exposure to recreational water
    a. family swimming pools
    b. public swimming pools
    c. lakes, rivers or seas
4. Personal hygiene
    a. hand-washing
    b. time of day hands were washed

Statistical analysis

Statistical analysis data was processed by the program EpiInfo 2002 ver.3.2 (CDC-WHO). The statistical analysis of the variables under study, as classified by socioeconomic level, was by the Chi square independence test and the Z test to compare two proportions. We estimated odds ratios with 95% confidence intervals (CI) [25].

Results

Study group characteristics

Ranking the schools according to socioeconomic distribution revealed that of the 56 public schools identified in the district, 12 (21%) belonged to the high level, 18 (32%) to the middle level, and 26 (46%) to the low level [24]. Based on the previously described sampling method, information was collected for all children present on the day of the survey-takers' visit in three schools from the high socioeconomic level (110 respondents), five schools in the middle level (394 respondents), and six schools in the low level (379 respondents) (Table 1). No significant differences were found among socioeconomic levels when comparing the distribution of schools sampled within the district ($p = 0.963$), the respondent groups ($p = 0.071$), or the two grades surveyed ($p = 0.065$).

Exposure to risk factors

Hamburger consumption: Of students surveyed, 94.6% (835/883) said they consumed hamburgers. Confidence intervals for types of hamburger consumed and the absolute and relative frequency of consumption are presented in Table 2.

Analysis of the data by socioeconomic status showed significant differences in the overall consumption of hamburgers, with the highest proportion in the high and middle levels ($p = 0.001$). There was no significant difference ($p = 0.11$) in the types of hamburgers consumed among the socioeconomic groups (Table 2).

Drinking water**:** Proportions of water consumption by socioeconomic strata were as follows: in students from the high-level socioeconomic group, 95 of 110 (87.0%) students reported drinking water, 350 of 394 (89.0%) in middle group, and 359 of 379(94.0%) in the low-level group (Table 3). The analysis of water consumption by socioeconomic status showed no significant differences ($p = 0.08$). Fifty-one (51) out of 804 (6.3%) who consumed water reported that the source of the water was from a well. Three hundred fifty-four out of the 804 (44.0%) students who consumed water also consumed other types of drinks: 276/354 (77.9%) soda, 33/354 (9.3%) fruit juices, 37/354 (10.5%) both soda and fruit juices, and 8/354 (2.3%) miscellaneous types of drinks (Table 4). Fifty-six students reported not consuming water, specifying other type of drink: 34/56 (60.7%) soda, 1/56 (1.8%) juice, 3/56 (5.4%) both soda and juice, and 18/56 (32.1%) several other drinks (the last three values were included as other in the table). Of all 883 students, 23 (2.7%) did not indicate their drink consumption patterns.

Recreational water: Analysis of exposure to recreational water showed that 853/883 (96.6%) of the children spent time swimming. A total of 636/853 (74.6%) respondents reported that they used family swimming pools, 296/853 (34.7%) used public swimming pools, 255/853 (29.9%) swam in the sea, 156/853 (18.3%) swam in rivers, and 42/853 (4.9%) swam in lakes (Table 5). Differences between children going to private/family swimming pools from different socioeconomic groups were not significant (p=0. 010). However, 47% of children from the middle and high-level socioeconomic groups who use family swimming pools (n: 354) also move to the sea during school holidays (n: 168) (p = 0. 000).

Personal hygiene: Of the children surveyed, 815/883 (92.3%) washed their hands while 68/883 (7.7%) did not. Time of hand-washing is detailed in Table 6. In addition, 499/883 (56.5%) of respondents reported that they never washed their hands before eating. Children from low socioeconomic levels washed their hands more frequently than those from other socio-economic groups did (p = 0.000). Out of 379 students from the low level, 206 (54.0%) reported washing their hands regularly as compared to 31 of 110 (28.0%) and 147 of 394 (37.0%) from the high and medium-level groups, respectively. The odds ratio for washing hands before eating was 36.02 (CI 4.94-262.64).

Discussion

This study evaluated exposure of schoolchildren population to known risk factors for developing HUS [9]. The study group was comprised of children up to 15 years old represent one of the populations known to be at high risk [6,15,17].

One risk factor strongly associated with HUS is meat consumption, particularly ground meat. A previous study using a case-control design showed an association between infection with STEC O157 and consumption of incompletely cooked hamburgers in the week prior to illness [26]. This study showed that 94% of children surveyed consumed hamburgers from a variety of sources. Differences among socioeconomic strata were in the frequency of consumption and not in the method of preparation.

In the last two generations, the consumption of precooked food has become widespread due to convenience, affordability, palatability and strong advertisement. This has resulted in substantial changes in handling methods for ground meat that place consumers at higher risk of exposure to undercooked meat. Changes in food handling such as inadequate cooking can contribute to the survival of pathogens in the meat with associated development of food-borne diseases. In Argentina, where meat consumption is estimated to be 63 kg/person/year [27], consumption of fast food increases the risk of exposure to pathogens, including STEC.

In the present study, nearly 80% of children consumed commercially prepared precooked hamburgers that were sold at stores regulated by the agency for Hazard Analysis and Critical Control Point (HACCP) and evaluated by health authority activities. Large numbers of children (60%) from all socioeconomic groups also reported consumption of homemade hamburgers. Meat handling and preservation methods at home can also contribute to the risk of cross-contamination with an associated increase in the number of pathogenic organisms in the meat [9,28]. Preparation methods of homemade hamburgers would naturally vary among different homes. Therefore, the role of the government in educating the general public in proper food handling is crucial to reducing the risk of meat contamination in the target population. The Argentine Food Code legislation bans the sale of previously ground beef in retail stores [29]. However, the beneficial effects of this law depend on the efficiency of meat inspection by regulatory authorities [27].

The population studied showed minimal exposure to untreated drinking water. Only 6.3% consumed well water that could be of poor quality and represent potential risk. Consumption of other beverages consisted primarily of soft drinks that do not carry an associated risk for HUS. A smaller percentage of children reported consumption of juices. Consumption of commercially prepared non-pasteurized apple juice has been associated with cases of HUS in the United States [30]. In Argentina, commercial juices are pasteurized and should not represent a potential source of contamination.

In Argentina, some cases of HUS are associated with contaminated recreational water [13,31]. Children generally play for long periods of time in recreational waters and are more likely to intentionally or accidentally swallow water [32]. The current study group showed high exposure to family-owned swimming pools. Although initial water quality may be safe, inadequate maintenance of pools represents a potential risk factor. Thus implementation of adequate chlorination protocols is necessary to reduce disease associated with contaminated pond water. Educating individuals regarding proper pool maintenance and its role in disease prevention is critical. In high and medium strata, this probable risk situation is reduced to the half in holyday, when children break to move to the sea. Moreover, fewer children in low strata use the

Table 1. Distribution of schools and students surveyed according to socioeconomic level

Group	Socioeconomic groups[a]			Total
	High	Medium	Low	
Public schools identified in each level	12 (21.4%)	18 (32.2%)	26 (46.4%)	56
Public schools sampled	3 (21.4%)	5 (35.7%)	6 (42.9%)	14
Students surveyed	110 (12.5%)	394 (44.6%)	379 (42.9%)	883
Students from 5th	63 (13.4%)	186 (39.6%)	221 (47.0%)	470
Students from 6th	47 (11.4%)	208 (50.4%)	158 (38.2%)	413

[a] No significant difference between socioeconomic groups

Table 2. Hamburger consumption in students aged 10-12 years from different socioeconomic levels

Hamburgers Consumption	N	Percent (%) Confidence Interval (Ci)	Source of Hamburguer[a]	Socioeconomic Groups			Total	Percent (%) Confidence Interval (Ci)
				High (n:110)	Medium (n:394)	Low (n:379)		
Yes	835	94.6 (93.0 - 96.1)	Homemade [b]	66	242	221	529	63.3 (60.0-66.7)
			Supermarket [b]	61	246	205	512	61.3 (57.9 - 64.7)
			Butcher [b]	61	218	187	466	55.8 (52.4 - 59.2)
			Cooked meals stores [c]	80	333	290	703	84.2 (81.6 - 86.7)
No	32	3.7 (2.6 -5.2)	-	-	-	-	-	-
NR	16	1.7 (0.9 -2.9)	-	-	-	-	-	-
Total	883	100	-	-	-	-	-	-

NR: No response
[a] Frequencies considered on multiple responses
[b] Considered as buying raw
[c] Bought cooked for consumption

Table 3. Consumption of water and source in different socioeconomic strata

Water Consumption	N	Percent (%) Confidence Interval (CI)	Type of Water	Socioeconomic Groups		
				High	Medium	Low
Yes[a]	804	91.0 (89.0-93.0)	From well	3	25	23
			Treated drinking water	92	325	336
No	56	6.4 (4.6-8.0)	-	11	28	17
NR	23	2.6 (1.4-3.7)	-	4	16	3
Total	883	100		110	394	379

[a] No significant difference between socioeconomic groups.

Table 4. Combinations of drink consumption in different socioeconomic strata

Drinks Consumption	N	Percent (%) Confidence Interval (CI)	Combination	Socioeconomic Groups		
				High	Medium	Low
Only water	450	55.9 (52.5-59.5)	-	54	201	195
Water & other drinks	354	44.1 (40.5-47.5)	water & soda	31	118	127
			water & juice	5	12	16
			water, soda & juice	5	14	18
			water & miscellaneous	0	5	3
Other drinks excluding water	56	67.8 (54.7-80.9)	soda	2	17	15
			other[a]	9	11	2
NR	23			4	16	3
Total	883			110	350	359

[a]: Situations not covered by the previous options with low frequency (≤ 3).
NR: No response

Table 5. Recreational water used according to socioeconomic level

Recreational water	Socioeconomic groups			Total
	High (n:110)	Medium (n:394)	Low (n:379)	(n: 883)
Number of respondents [b] (proportion)	**104** (94.5%)	**383** (97.2%)	**366** (96.5%)	**853** (96.6%)
Type of recreational water [a]				
Family swimming pools [b]	75	279	282	636
Public swimming pools [b]	25	154	117	296
The sea	29	139	87	255
Rivers	20	65	71	156
Lakes	9	14	19	42

[a] Corresponds to multiple responses from the total survey
[b] No significant difference between socioeconomic groups.

Table 6. Hand-washing patterns and frequency in schoolchildren related to socioeconomic level

When hands were washed	Socioeconomic groups			Total (%)[a]
	High (n:110)	Medium (n:394)	Low (n:379)	(n: 883)
Before eating	31	147	206	384 (43.5)
After toileting	19	121	127	267 (30.2)
Morning-Afternoon and Evening	32	104	71	207 (23.4)
On returning home	7	38	29	74 (8.4)
After eating	10	28	33	71 (8.0)
After touching something dirty	2	31	22	55 (6.2)
When cooking	4	19	11	34 (3.8)
In the morning	0	5	6	11 (1.2)
Other [b]	10	26	17	53 (6.0)

[a] Corresponds to multiple responses from the total survey
[b] Situations not covered by the previous options and low frequency (≤ 3).

recreational open water and therefore depend on the sanitary quality of the pools. Public swimming pools depended on current required regulatory inspections by qualified authorities to ensure water quality. The positive impact of such regulatory measures on health and safety has been previously documented [33,34].

The importance of hygiene measures, particularly hand-washing, in the prevention of disease, including acute infantile diarrhoea, has been previously documented [34]. In Argentina, hand-washing has been identified as a protective factor in a multivariate analysis for STEC infection [9]. Therefore, in the current study, personal hygiene habits were assessed based on frequency and time of hand-washing. In the present study, schoolchildren generally practiced hand-washing but many of them did not wash their hands prior to eating food. Since hand-washing before eating is critical to the prevention of food-borne diseases, those who fail to wash their hands before eating are at risk of exposure.

Hand-washing should be considered an important preventive measure and strongly encouraged. However, most of the schools visited did not have the necessary elements of hygiene, such as soap in the bathrooms. Behavioral changes in these groups are unlikely without adequate infrastructure and appropriate institutional support.

All the identified risks are preventable through appropriate health and consumer education of children and their families. Consumer demand for a safe food supply must be addressed with an institutional framework maintained under governmental supervision. The government of Argentina has sporadically undertaken such prevention campaigns, but these measures have not demonstrated long-term impact on the incidence of disease. Short-term positive results, such as the decreased incidence of infant diarrhoea during the campaign to prevent cholera disease (Morales R, unpublished results, 2006), are occasionally reported. Unfortunately, none of these campaigns have resulted in lasting behavioral changes.

Given the endemic nature of HUS in Argentina, isolated and local campaigns are unlikely to reduce incidence of the disease. Implementation and sustainment of a national program for prevention and control will provide the greatest benefit to the general population. Health education programs should be directed at increasing awareness of food safety and adequate personal hygiene. The educational concepts that reduce the incidence of diarrhoea in developing countries are outlined in strategies promoted by the Pan-American Health Organization (PAHO) and the World Health Organization (WHO) [35].

Encouraging individuals to take positive measures for improving food safety and hygiene measures is highly desirable and critical in the prevention of food-borne diseases. Reduction of risk also depends on cultural dietary changes that will help decrease the burden of infectious disease in developing countries [21].

More than 200 food-borne diseases with high morbidity and mortality rates in children are currently recognized. It is imperative to develop coordinated and integrated actions to decrease illness associated with these diseases. These illnesses also contribute to increased costs within the health-care system, have a strong social impact, and create distrust in the community regarding safety in the food chain. In particular, treatment costs associated with HUS significantly exceed the investment in prevention [8]. We contend that focusing on health risks is key to prevention of disease. Correctly implemented, high-quality health programs that do not discriminate based on cultural and socioeconomic conditions will improve overall child health [36]. Such risk reductions and sustainable development may also help in reducing inequalities in the society. These changes are not only the responsibility of governments but the concern of all individuals within the population [21]. The current study demonstrates that Argentinian children are at high risk of exposure to known risk factors for the development of HUS. These findings confirm the need for improved educational programs targeted at improving personal hygiene, establishment of correct meat handling and cooking techniques, and maintenance of safe recreational water. Further studies are needed to identify appropriate educational methods targeted at different cultural and socioeconomic groups in the prevention food-borne disease.

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