Family Clusters of Shiga Toxin-producing *Escherichia coli* Infection

An Overlooked Source of Transmission. Data From the ItalKid-Hus Network

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Background: The aim of the present work was to investigate family clusters of Shiga toxin-producing Escherichia coli (STEC) infection among the household members of STEC positive patients, identified within a screening program of bloody diarrhea (BD) for STEC in Northern Italy.

Methods: Stool samples from patients with BD or BD-associated-hemolytic uremic syndrome (HUS) and related households were investigated by molecular and bacteriologic methods to detect and characterize the virulence profile of STEC and Pulsed Field Gel Electrophoresis analysis were done on isolates.

Results: Thirty-nine cases of STEC infection (isolated BD in 16, BD-associated-HUS in 23) were considered, and a total of 130 stool samples from 1 to 8 households of the index patient were analyzed. The prevalence of positivity was higher in siblings (34.8%, 8/23) than in mothers (20%, 7/35), grandparents (9.5%, 2/21), fathers (8.8%, 3/34) or other households. In 14 clusters (36%), one or more household shared a STEC with the same virulence profile (*stx*, *eae*, serogroup) as the index case. In 7 clusters, STEC strains isolated from at least 2 subjects also shared identical Pulsed Field Gel Electrophoresis profile. The frequency of household infection does not appear to be associated to the index case's illness (HUS or BD), nor with the serotype or with the virulence profile of the involved STEC (*stx2* or *stx1-stx2*).

Conclusions: Our study shows that STEC infections, most likely related to human-to-human transmission, are common among households of patients with STEC BD or HUS and underlines the importance of extending the epidemiologic investigations to all family members, as the index case may not always be the primary infection in the family.

Key Words: Shiga toxin-producing *Escherichia coli*, STEC infection, household transmission

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Shiga toxin-producing Escherichia coli (STEC) are responsible for gastroenteritis, often bloody, due to their ability to produce Shiga toxin 1 (Stx1) or Shiga toxin 2 (Stx2), encoded by the *stx1* and *stx2* genes, respectively.

Virulent strains of STEC very commonly carry genes encoding other virulence factors such as intimine (eae) and mainly belong to a small group of serogroups, among which O157 and O26 are the most frequent ones, together with O103, O145 and O111 (the Top five).[1] STEC-associated diarrhea can have complications, including hemolytic uremic syndrome (HUS), which develops in about 5% to 10% of patients. The virulence profile of the strains and the patient's age represent the main HUS risk factors for the development of the renal complication.[2] The origin of STEC infections is commonly attributed to the consumption of contaminated food or water or to close contacts with carrier animals such as cattle, sheep or goats.[1] Interhuman transmission is reported in the literature, mainly in the context of outbreaks of infection occurring in day-care centers where the contact between children is high. In the setting of outbreaks and more rarely in sporadic infections, secondary cases of infection in household contacts have been previously described.[3–10] Despite the considerable efforts being made, at any level, by departments dedicated to the prevention of communicable diseases, aimed at identifying the source of STEC infection, these investigations are rarely successful, and in the vast majority of cases, the source goes unidentified. STEC infection can be paucisymptomatic or asymptomatic, especially in adults, nevertheless infected subjects can eliminate the microorganism with stools for a long time ranging from few days, weeks or several months.[5,10–12]

In Northern Italy, a centralized screening program of bloody diarrhea (BD) for Stx in children aimed to the early identification, referral, and inpatient management of STEC infected children at high risk of developing eHUS, has been active since 2010. In this context, it was decided to investigate cross-contamination of subjects within families and to consider the epidemiologic implications related to the origin and transmission of STEC infection.

MATERIALS AND METHODS

Study Design

A network devoted to the screening for Stx of BD in children (age <20 years) was developed in 2010 by the Center for HUS Prevention Control and Management at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico on Milan (Italy), aimed at early diagnose and manage STEC HUS. The network (Italkid-HUS Network) connects 63 pediatric units in Northern Italy (referral general population: 12 million; 2.3 million children). From July 19, 2016, to September 12, 2019, as part of the surveillance system, the screening was extended to the available household contacts of patients found Stx+ (index case). The following information was

collected for the index case and for the tested household contacts: age, sex, sampling date and the relationship with the index case.

Bacteriologic and Molecular Analysis

The fecal specimens were collected through the BD surveillance network and, on arrival at the central laboratory, they were directly streaked onto Mac Conkey Agar (MCA1) and fecal swabs were resuspended in Mac Conkey Broth (MCB). After an overnight incubation at 37°C, MCB was plated onto a second Mac Conkey Agar (MCA2) and was tested for the presence of *stx1*, *stx2* and *eae* genes by Reverse Dot Blot assay (Genotype enterohemorrhagic *E. coli*-Arnika, Hain Lifescience GmbH, Nehren, Germany) between 2010 and 2017. From 2018 the tests were performed by multiplex real-time polymerase chain reaction (PCR) using the RIDA-Gene enterohemorrhagic *E. coli*/enteropathogenic *E. coli* kit, confirmed by the RIDA-Gene *E. coli* Stool Panel I kit (R-biopharm, Darmstadt, Germany). In case of positivity for *stx* genes, MCB samples and the related colonies on MCA1 were subjected to further molecular analysis and tried for STEC isolation according to the EU-RL VTEC method[13] adapted to clinical samples as briefly described. DNA extracted from MCB and MCA1 cultures (mixed colonies) were subjected to real-time multiplex PCR for the serogroups most frequently associated with human infection: O157, O26, O103, O111, O145, O111 plus O104, responsible for the German epidemic occurred in 2011 (Top5 + O104). This step generated a STEC positivity of the samples based on positive signals in PCR of a given profile (*stx*, *eae* and serogroup specific genes) in enriched cultures.

The isolation attempts were concluded by testing up to 50 colonies obtained partly from MCA1 and partly from MCA2 and including also non lactose-fermenting colonies if present. Pooled colonies DNA (pool of 10) was retested for *stx1*, *stx2*, *eae* and for the 6 serogroups considered. Finally, single *stx* positive colonies with a given virulence profile generated as many strains, were subjected to Pulsed Field Gel Electrophoresis (PFGE), according to Ribot et al.[14] The subtyping of the selected strains was performed in accordance with the PulseNet protocol, using XbaI as the restriction enzyme and the analysis of the pulsotypes obtained was carried out using the BioNumerics software (V. 7.5, Applied Maths, Sint-Martens-Latem, Belgium).

Statistical Analysis

Prevalence and confidence intervals (CIs) at 95% were provided for different groups and Fischer's exact test was performed to analyze the differences. The results are reported as odds ratios with 95% CIs and 2-tailed *P* values. A *P* value of ≤ 0.05 was considered statistically significant. All analysis was performed by GraphPad Prism 8.01 (GraphPad Software Inc. San Diego, CA).

RESULTS

Thirty-nine families of STEC infection cases were identified and considered for the present analysis of 130 stool samples from households of the index cases. In 23 index cases, the primary infection had been complicated by STEC HUS, while the remaining 16 index cases only exhibited BD. All index cases were associated with Stx2-producing STEC (in 14 cases combined with Stx1). Serogroup O157 was involved in 28.2% (11/39) of the index cases, followed by O26, O103 and O111 in 7 (17.9%), 4 and 4 cases (10.2%), respectively. "non Top5+O104" STEC were involved in the remaining 13 index cases (33.3%) (Table 1).

In 14 cases (35.9%), at least one additional household contact was positive for STEC, and the same virulence profile (*stx*, *eae*, serogroup) as the index case was documented in 12 of the 14 families by positive PCR signals detected from stool samples. In fact, 2 HUS index case were STEC negative, but one or more household contact was positive for Stx (the mother in one case and both the mother and the sister in the second).

As far as the relationship with the index case, in as many as 8 clusters, the additional positive household contacts were siblings (5 sisters and 3 brothers), in 7 clusters mothers were involved while in 3 the father and in 2 the grandparents were positive. Altogether, 20 households of 130 investigated (15.4%) were found positive. The highest rate of positivity was found among the 23 investigated sibling (34.8%) and 35 mothers (20%). Fathers (n: 34) and grandparents (n: 21) showed a rate of 8.8% and 9.5%, respectively. Prevalence of STEC positivity was significantly higher in siblings compared with fathers [$P = 0.016$ (odds ratio, 5.5; 95% CI, 1.3–20.7)] or other households ($P = 0.003$), while differences between other groups were not significant. None of the other household contacts sampled (n = 17) tested positive (Table 2).

In 7 clusters of the 14, STEC of a given profile (*stx*, *eae*, serogroup) were isolated from at least 2 subjects, allowing for comparison of strains through molecular analysis. The PFGE profiles of such strains were determined and, in all cases, they were identical within the cluster (Table 3).

Clinical information on household contacts found positive were not systematically recorded. However, mild diarrhea, nausea or dyspepsia have been frequently reported but none of the household contacts developed secondary HUS.

DISCUSSION

The results of the present study show that a significant number (14/39, 36%) of sporadic STEC infection are associated with STEC infection in household contacts of the index case. This evidence is based on the STEC detection by multiplex PCR of *stx*, *eae*

TABLE 1. Number of Families With Positive Households in Relation to Those Investigated for Each Variable Considered: Case Patient Disease, *STX* and Serogroup of the Involved STEC

Variable	Type	N. Index Cases	N. Families With Positive Households	Prevalence %	95% CI	*P* Value
Condition	HUS	23	9	39.1	(22.2–59.2)	Ns
	BD	16	5	31.3	(14.2–55.6)	
stx	*stx2*	25	10	40.0	(23.4–59.3)	Ns
	stx2, *stx1*	14	4	28.6	(11.7–54.6)	
Serogroup	Top5 + O104*	26	9	34.6	(19.4–53.8)	Ns
	Non-Top5 + O104	13	5	38.5	(17.7–64.5)	
Total		39	14	35.9	(22.7–51.6)	

*Top5 = O157, O26, O103, O111 and O145.

Prevalence, confidence intervals at 95% and results of Fischer's exact test between groups.

TABLE 2. Number of Family Members Investigated and Number of STEC Positive per Type of Relationship in 39 Families, 14 of Which With at Least 1 Positive Family Member (35.9%)

Type of Household	Total Investigated	N. of Positive	Prevalence %	95% CI
Siblings	23	8	34.8	(18.8–55.1)
Mothers	35	7	20.0	(10–35.9)
Fathers	34	3	8.8	(3–23)
Grandparents	21	2	9.5	(1.7–28.9)
Other	17	0	0.0	(0–18.4)
Total	130	20	15.4	(10.2–22.6)

Prevalence and confidence intervals at 95% for type of household.

and serogroup specific genes of similar profile in 2 or more household contacts of an index case and on confirmation in part of the clusters (when at least 2 isolates from in the same cluster were obtained) of the PFGE identity of the involved strains. Although the standard PFGE performed with the Xbal enzyme alone can be sometimes prone to type II error[15] (identical profile in isolates that are actually unrelated), we believe this was not relevant in our setting. In fact, the PFGE performed in over 100 isolates within the activity of the ItalKid network (sporadic cases or family clusters from this study) showed a very high variability and no identical strains except those coming from the same family cluster (personal data).

The observed frequency of STEC family clusters can only be underestimated, given that the investigation was not systematically performed in all of the household contacts. Our results support the usefulness of screening household contacts given that 15.4% were positive; the identification of carriers, regardless of their symptoms, remains a cornerstone of primary prevention, by avoiding the intra-family and community transmission chain (ie, childcare centers personnel or food handlers), given also the severity of the complication.

In our family clusters, siblings of the index case were the most frequently positive among tested household contacts, followed by mothers and occasionally by fathers or grandmothers. It seems reasonable to hypothesize that the infections in household contacts were more likely due to person-to-person transmission, although exposure to the same contaminated food or to other common environmental risk factor causing a co-primary colonization cannot be excluded. Our study, besides investigating sporadic cases of HUS, also included patients with BD identified through the screening of BD for Stx being active in Northern Italy since 2010. The clinical condition of the index case (HUS or BD), the serogroup and virulence profile of the identified STEC strain did not influenced the likelihood of positivity among the household contacts.

In 2 clusters included in the present study, the index case, with overt HUS, was Stx negative, but the investigation of household contacts identified Stx positive subjects. This finding may be very important to establish the diagnosis of STEC-related HUS in the index case, as a negative STEC screening might induce clinicians to qualify the case as atypical HUS and to start the related specific therapeutic intervention (anti-C5 inhibition). Thus, the identification of one or more STEC positive household contact supports (or rules in) the diagnosis of typical HUS despite the (falsely) negative test for Stx in the index case.

The present study cannot provide detailed clinical information on household contacts found positive for Stx because these were not systematically recorded. However, mild diarrhea, nausea or dyspepsia have been frequently reported but none of the household contacts developed secondary HUS.

Furthermore, information regarding the timing of symptoms or of STEC clearance from stools in household contacts in comparison with the index case are not available. These data could have provided useful indications regarding the risk of interhuman transmission in families. However, given the demonstration that STEC can persist in stools for a long time, even in asymptomatic subjects, it can be speculated that the index case may have contracted the infection from an asymptomatic household contact.

Similar observations have been reported in 2 studies conducted in Argentina and Netherlands where STEC infection was reported in one or more household contacts of HUS patients with a frequency of 36% or 68% of the families, respectively.[3,16] In another

TABLE 3. Virulence Profile of STEC Involved in the 14 Clusters, Age and Clinical Condition of the Index Case, STEC Positive Household Contacts Tested and Found Positive by Multiplex PCR for *stx*, *eae* and Serogroup Specific Genes

Family	STEC Serogroup and Virulence Profile	Age of Index Case	HUS/BD	Tested Household Contacts	STEC Positive Household Contacts	Isolation and PFGE Identity Between Household Contacts
1/2016	Non Top6, *stx2, eae*	1	BD	Mo, Fa, GMo, GFa, Br	Ic, Br	Ic/Br
2/2016	O157, *stx2, eae*	2	BD	Mo, Fa, Sr, Gmo	Ic, Sr, Mo	Ic/Sr
3/2016	O157, *stx1*, *stx2, eae*	7	BD	Sr	Ic, Sr	Ic/Sr
4/2016	O157, *stx1*, *stx2, eae*	4	HUS	Sr	Ic, Sr	nd
1/2017	Non Top6, *stx2, eae*	1	HUS	Mo, Fa, Br, GMo (2), GFa, Oth (2)	Ic, Br	Ic/Br
11/2017	Non Top6, *stx2, eae*	4	HUS	Mo, Fa, GMo, Br (2)	Ic, Br	nd
15/2017	O111, *stx2, eae*	1	HUS	Sr	Ic, Sr	Ic/Sr
1/2018*	O157, *stx1*, *stx2, eae*	8	HUS	Mo, Fa, Sr	Sr, Mo	nd
2/2018	O26, *stx2, eae*	1	HUS	Mo, Fa, GMo, GFa	Ic, Mo, Fa, GFa	Ic/Fa
5/2018	O103, *stx1*, *stx2, eae*	2	HUS	Mo, Fa, GMo, GFa	Ic, Mo, GMo	Ic, Mo, GMo
7/2018	O26, *stx2, eae*	5	BD	Mo, Fa	Ic, Fa	nd
8/2018	Non Top6, *stx2*	9	BD	Mo, Fa, Br (2)	Ic, Mo, Fa	nd
10/2018*	Non Top6, *stx2, eae*	2	HUS	Mo, Fa, GMo, GFa, Oth (3)	Mo	nd
3/2019	O26, *stx2, eae*	<1	SEU	Mo, Fa, Sr	Ic, Mo	nd

*In these families the index case was STEC negative and the virulence profile of the cluster was determined by the STEC positivity of household contacts.

Br indicates brother; Fa, father; GFa, grandfather; GMo, grandmother; Ic, index case; Mo, mother; Oth, other household contacts; Sr, sister.

The last columns indicate the clusters were the STEC correlations were confirmed by the isolation of at least 2 strains in the same family with identical PFGE profile.

study considering sporadic O157 STEC infections the transmission rate to household contacts was calculated to be 15%, some of which showing diarrhea as early as 7 days before the index case and being children under 5 years of age particularly affected.[7]

Other reports already documented the transmission of STEC infection to household contacts from children being infected in childcare centers. For example, Tokuda et al,[9] showed a transmission rate of up to 34.4% in Japan, while in Scotland there were reported 11% of secondary cases of 228 O157 infections registered in a decade.[6] Similar data were reported in the United States for O26[10,17] and for O157 STEC infections.[5] Moreover, a meta-analysis study conducted on 90 outbreaks of O157 infection in 6 different states, concluded that 19% of registered cases were of secondary origin.[8] Secondary transmission was also documented during the severe STEC O104 epidemic that occurred in Germany in 2011,[18] although with lower frequency.

The frequent involvement of household contacts in STEC infections should reinforce the recommendations of hygiene preventive measures to avoid secondary infection and suggests that these recommendations should be given directly rather than by phone or letter.[9,19,20]

An additional issue raised by the finding of person-to-person STEC transmission within families is that the elimination of STEC continues in patients (for as long as several weeks or months) as documented by Scavia et al,[12] with STEC O26 in a nursery, in childcare centers in United States[5,10] or in a large study conducted in Sweden on patient over 10 years of age.[11]

For this reason, epidemiologic investigations aimed at identifying the source of infection, which are generally concentrated in tracing consumed foods or at-risk behaviors of the index case, are largely incomplete and almost invariably unsuccessful.

The interhuman transmission among household contacts is rarely investigated as a possible origin of the infection which might have been introduced in the family by an asymptomatic or paucisymptomatic STEC shedding subject.

In conclusion, our study reveals that secondary STEC infections of interhuman origin are common in household contacts of patients (particularly siblings and mothers). In this view, strict hygiene rules among household contacts of a STEC infected patient should be recommended to reduce the risk of transmission. The importance of extending the epidemiologic investigation to trace the origin of the infection to all household contacts, also emerges from the possibility of identifying other subjects (particularly siblings) who might require close monitoring and early treatments. Finally, the benefit might not be restricted to household contacts, but also extend to the community where potential carriers can constitute a hazardous source of infection.

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