Brief Original Article

First isolation in Argentina of a highly virulent Shiga toxin-producing *Escherichia coli* O145:NM from a domestic cat

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Abstract

Introduction: Hemolytic uremic syndrome (HUS) is distributed worldwide. In Argentina, more than 450 cases of HUS, mostly sporadic, are reported annually. The main serotype isolated is O157:H7, and among non-O157 STEC, O145:NM is the most frequent strain. We studied the relationship of companion animals living in contact with a child with sporadic HUS, as carriers of Shiga toxin-producing *Escherichia coli* (STEC) strains.
Methodology: Duplicate rectal swab samples were taken weekly from the household cat and dog at the home of a patient with HUS. Samples were plated on MacConkey and sorbitol MacConkey-CT agar. Confluent growth from each plate was screened for the presence of stx_1, stx_2 and rfb_{O157} gene by PCR assays. Up to 300 individual colonies taken from positive plates at screening were retested by PCR.
Results: The strain from the cat belonged to the highly virulent serotype O145:NM. Although this strain differed antigenically from the strain isolated from a child with HUS living in the same house, both carried the stx_2, *eae* and *ehx*A virulence genes. The strain isolated from the dog belonged to the serotype O178:H19.
Conclusions: An asymptomatic household cat may harbour the high virulent STEC strain, such as O145:NM, the second most frequently STEC serotype associated with HUS in Argentina. Companion animals are probably exposed to the same sources as the humans. More studies are needed to establish dogs and cats as sources of infection in the epidemiological cycle of infections caused by STEC strains, and to develop effective control strategies for this pathogen.

Key words: STEC; HUS; cat; pet

Introduction

Hemolytic uremic syndrome (HUS) is widely distributed around the world. In Argentina, more than 450 cases of HUS are reported annually, and in the last five years the notification rate has increased by 1.7% [1]. HUS is the main cause of acute renal failure and is responsible for 20% of the renal transplants among children in this country [2]. Humans frequently become infected with Shiga toxin-producing *Escherichia coli* (STEC) by the ingestion of contaminated food or water, person-to-person transmission, or direct contact with animals that represent a threat for STEC infection (*e.g.*, farm animals, ruminants) [3,4,5]. Food has been identified worldwide as the source of infection in approximately half of the cases of HUS [6]. Despite that ruminants in general and cattle in particular have been implicated as the main STEC reservoirs [7], other animals can also maintain the strains in the environment [8,9]. A carrier rate of *E. coli* O157:H7 in companion animals was recently described by Kataoka *et al*. in Japan [10]. The aim of this study was to evaluate the relationship of household pets, a cat and a dog, living in contact with a child with HUS, as carriers of Shiga toxin-producing *Escherichia coli* (STEC) strains and their potential role in the epidemiological cycle of STEC infection.

Methodology

Ethical considerations

The pets were handled under the supervision of licensed veterinarians according to standards set by the Institutional Ethics Committee, Faculty of

Veterinary Science, University of Buenos Aires, Argentina.

Patient

The patient was an 11-year-old boy with diagnosis of HUS, living with his parents and a 14-year-old brother in an apartment of Buenos Aires City, Argentina. Ingestion of raw/undercooked meat, raw milk, and contact with farm animals were excluded. Virulence characteristics of the O157:H7 strain (stx_2, *eae*, *ehx*A) isolated from the patient, by methods described elsewhere [11], were reported by the service Fisipopatogenia, Instituto Nacional de Enfermedades Infecciosas, ANLIS "Dr C.G. Malbrán "Buenos Aires, Argentina (2007). One dog and one cat were the companion animals of the family.

Companion animals

Both animals lived in close contact with the owners, usually climbing on the tables and beds. They were fed only with commercial food, and contact with other animals was excluded.

Sample collection

Duplicate rectal swab samples were taken weekly from the household pets at the home of a patient with HUS. The first samples were collected during the same week of HUS diagnosis (on day 4 after the HUS confirmation). Then samples were weekly collected (days 11, 18, 25, 32 and 39). Sampling was continued until two successive negative results were found for both animals.

Enrichment and plating procedures

Samples, collected in Stuart's medium, were processed the day of collection by inoculating culture tubes with tryptic soy broth (TSB) and tryptic soy broth plus tellurite and cefixime (TSB-CT). After 6 hours of incubation at 37ºC and 42ºC respectively in aerobiosis, cultures from TSB were streaked onto MacConkey agar (MAC), and those from TSB-CT onto sorbitol MacConkey-CT agar (SMAC-CT) plates and incubated in aerobiosis at 37ºC for 18 hours.

Screening for STEC strains by PCR assays

E. coli strains ATCC 25922 (stx_1^-, stx_2^-, *eae*⁻, *saa*⁻, *hly*A⁻, and *rfb*O157⁻), EDL 933 (O157:H7, stx_1^+, stx_2^+, *eae*⁺, *hly*A⁺ and *rfb*O157⁺), UNCPBA O91:H21 (stx_1^+, stx_2^+, *eae*⁻, *saa*⁺, *hly*A⁺ and *rfb*O157⁻) were used as controls. Screening for stx_1, stx_2 and rfb_{O157} genes was performed by PCR as previously

described [12], from the confluence zone of MAC and SMAC-CT plates as template [13]. From each positive plate at screening the presence of the genes was investigated in up to 300 CFU through pools with up to ten colonies. Individual colonies of each positive pool were re-tested by PCR. For further identification, all positive colonies to any of the genes under study were streaked on tryptic soy agar plates.

Characterization of STEC strains

All strains were confirmed as *E. coli* by biochemical tests as previously described [13] and the enterohemolytic activity was determined using washed sheep blood cells agar supplemented with calcium according to Beutin *et al*. [14]. Identification of somatic (O) and flagellar (H) antigens was performed following standard methods of tube agglutination test [15] and using currently available O (O1 to O181) and H (H1 to H56) antisera as described elsewhere [16]. Antimicrobial susceptibility patterns were determined according to CLSI standards [17], and the following antimicrobials were used: nalidixic acid, 30 μg; ampicillin, 10 μg; ciprofloxacin, 5 μg; chloramphenicol, 30 μg; streptomycin, 10 μg; gentamicin, 10 μg, nitrofurantoin, 300 μg; tetracycline, 30 μg; and trimethoprim-sulfamethoxazole, 25μg. Testing conditions by disk diffusion agar and results were evaluated according to CLSI [17,18]. All STEC strains carrying the stx_2 sequence were tested by PCR for the presence of *eae, saa* and *ehx*A virulence genes (Table 1). Primers and PCR conditions were those described previously [13,19,20]. Genotyping of stx_2 variants was performed by RFLP-PCR according to previous reports using primers VT2-c/VT2-d and VT2v-1/VT2v-2 [21], VT2-e/VT2-f [22], SLTv-IIvc/CKS-2 [23] (Table 1).

Results and discussion

The first sample taken from the cat during the week of detection of the HUS case (day 4) was positive for the stx_2 gene, but the sample collected from the dog was negative (Table 2). On days 18 and 25, samples from the dog were positive for the stx_2 gene. On days 11, 32 and 39, samples from both animals were negative in the screening and therefore samplings were stopped. Both animals were asymptomatic during the time of sampling. The STEC strains isolated from the cat on day 4 belonged to the serotype O145:NM. The genetic profile of the

Table 1. Primers, DNA sequences, target genes, conditions, product sizes and references of PCR used in the study

Primer	Sequence (5' - 3')	Target	Condition	Product size (bp)	Reference
Stx1R Stx1F	AGCGATGCAGCTATTAATAA GAAGAGTCCGTGGGATTACG	stx_1	94 ℃ 30 s 58 ℃ 30 s 72 ℃ 30 s 30 cycles	130	[12]
Stx2FC Stx2R	TTAACCACACCCCACCGGGCAGT GCTCTGGATGCATCTCTGGT	stx_2	94 ℃ 30 s 58 ℃ 30 s 72 ℃ 30 s 30 cycles	346	[12]
rfbO157f rfbO157r	CGGACATCCATGTGATATGG TTGCCTATGTACAGCTAATCC	*rfbO157*	94℃ 30 s 58 ℃ 30 s 72 ℃ 30 s 30 cycles	259	[12]
eae1 eae2	GGAACGGCAGAGGTTAATCTGCAG GGCGCTCATCATAGTCTTTC	*eae*	94 ℃ 45 s 62 ℃ 30 s 72 ℃ 30 s 30 cycles	346	[13]
saaDF saaDR	CGTGATGAACAGGCTATTGC ATGGACATGCCTGTGGCAAC	*saa*	95 ℃ 1 min 65 ℃ 2 min (10 cycles) decrementing to 60°C 2min by cycle 15 60°C 2min 72°C 2.5min (10 cycles)	119	[19]
ehxA1 ehxA2	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGGTTAAGCT	*ehxA*	95 ℃ 1 min 65 ℃ 2 min (10 cycles) decrementing to 60°C 2min by cycle 15 60°C 2min 72°C 2.5min (10 cycles)	534	[20]
VT2-c VT2-d	AAGAAGATGTTTATGGCGGT CACGAATCAGGTTATGCCTC	stx_2, $stx_{2(vh-a)}$ $stx_{2(vh-b)}$	94 ℃ 2 min 55 ℃ 2 min 72 ℃ 1 min 30 cycles	285	[21]
VT2v-1 VT2v-2	CATTCAGAGTAAAAGTGGCC GGGTGCCTCCCGGTGAGTTC	$stx_{2(vh-a)}$ $stx_{2(vh-b)}$ $stx_{2d-Ount}$ $stx_{2d-OX3a}$	94 ℃ 2 min 55 ℃ 2 min 72 ℃ 1 min 30 cycles	385	[21]
VT2-e VT2-f	AATACATTATGGGAAAGTAATA TAAACTGCACTTCAGCAAAT	stx_2 $stx_{2(vh-a)}$ $stx_{2(vh-b)}$ $stx_{2d-Ount}$ $stx_{2d-OX3a}$	94 ℃ 25 s 55 ℃ 50 s 72 ℃ 26 s 30 cycles	348	[22]
SLTvIIvc CKS2	ACCACTCTGCAACGTGTCGC ACTGAATTGTGACACAGATTA	stx_{2d} activatable	94 ℃ 1 min 56 ℃ 1 min 72 ℃ 1 min 30 cycles	890	[23]

Table 2. Sampling time, screening and profile of STEC strains isolated from household pets related with a sporadic case of HUS

Sampling Days [1]	Screening for stx_1/stx_2		Isolated serotype	Virulence profile					*stx* genotype
	dog	cat		stx_1	stx_2	*eae*	*ehxA*	*saa*	
4	-	+	O145:HNM	-	+	+	+	-	stx_2
11	-	-	-						
18	+	-	O178:H19	-	+	-	-	-	$stx_{2(vha)}$
25	+	-	-						
32	-	-	-						
39	-	-	-						

(1)   Day 0: child diagnosis of HUS

isolates corresponded to $stx_2{}^+$ eae^+ $ehxA^+$. This strain belonged to the genotype stx_2 as determined by RFLP-PCR, and exhibited the enterohemolytic phenotype.

We were able to isolate the STEC 0178:H19 strain from the dog on day 18. The strain carried the $stx_{2(vh-a)}$ gene; however, it was neither enterohemolytic nor carrying *eae* and *saa* genes. Despite the positive result on screening, no STEC isolates were recovered from the sample collected from the dog on day 25. All STEC isolates were susceptible to all antibiotics tested.

In Argentina, approximately 60% of HUS cases are due to O157:H7, and among the non-O157 STEC serotypes, O145:NM is the most frequent strain [24]. Although a high virulent O145:NM STEC serotype had been isolated from the household cat in the week of the detection of the HUS case, an O157:H7 strain was isolated from the child presenting with clinical signs of HUS.

Since screening for STEC in human samples was stopped when an STEC strain was recovered, due to the limitation of the methodology [25], one cannot exclude the possibility of isolating more than one STEC serotype from the patient with HUS and from the cat as well. In this patient case, we did not screen specifically for the O145:NM serotype. Although immunomagnetic separation-based detection of O157 is routinely used in human samples, IMS-based detection of serotypes other than O157 were not available in 2007. Moreover, IMS to O157 strains was not performed in the pets' samples.

Conclusion

A close relationship between children and their pets was seen in the city of Buenos Aires, Argentina [26]. Although it is not possible to determine the source of infection in this home, and any of the strains from the companion animals were directly associated with the sporadic case of HUS, the isolation of STEC strains in both animals from the same home is striking, especially given the local prevalence previously determined for these species which was as low as 1.1% (5/450) in dogs, and 2.6% (4/149) in cats [13]. Virulent STEC strains may be harboured by asymptomatic household dogs and cats, which are probably exposed to the same sources as the humans. More studies are needed to establish companion animals as a source of infection or accidental carriers of STEC in the epidemiological cycle of infections caused by STEC strains, and to develop effective control strategies for this pathogen. The isolation of O145:NM from a cat has already been documented in Germany [9]. This strain was also isolated from cattle in Argentina [27], but this is the first time that this serotype with an enterohemorrhagic *E. coli* virulence repertoire has been recovered from a domestic cat in our country. The potential role of dogs and cats in the epidemiological cycle of STEC human infection is still poorly investigated. Due to the close cohabitation between companion animals and their owners, the transmission of sporadic cases of HUS is probable.

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