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BRIEF REPORT

Shiga Toxin–Producing *Escherichia coli* Transmission via Fecal Microbiota Transplant

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(See the Editorial Commentary by Khanna and Kraft on pages e881–2.)

Fecal microbiota transplantation (FMT) is recommended therapy for multiply recurrent *Clostridioides difficile* infection. We report adverse events in 7 patients who received FMT from a stool donor who was colonized with Shiga toxin–producing *Escherichia coli* (STEC). No patients died of FMT-transmitted STEC. Improved screening can likely avoid future transmission.

Keywords. fecal microbiota transplantation; FMT; Shiga toxin-producing *E. coli*; STEC; *Clostridioides difficile*.

Fecal microbiota transplantation (FMT), the transfer of donor stool into the gastrointestinal tract of a patient, is a recommended treatment for multiply recurrent *Clostridioides difficile* infection (CDI) [1–4]. Transmission of virulent organisms, a known risk of FMT, is partially mitigated by careful donor screening and patient surveillance [5]. Because of FMT safety practices, there have been few reported transmissions of infections in placebo-controlled or observational cohort studies to date [6, 7]. The importance of donor screening was recently highlighted by the first case series of an infection due to FMT [8], which subsequently led to improvements in donor screening best practices for the microbial therapeutics field [9].

Here, we report the transmission of Shiga toxin–producing *Escherichia coli* (STEC), confirmed by genomic sequencing, via FMT produced by a nonprofit stool bank (OpenBiome, Cambridge, MA). These adverse events were included in a safety alert issued by the US Food and Drug Administration (FDA) in March 2020 [10, 11]. We describe the surveillance activities that detected these adverse events, the results of an investigation, and the risk-mitigation activities implemented, including recall of implicated material.

DONOR SCREENING, MATERIAL PREPARATION, AND SURVEILLANCE

At the stool bank that produced the implicated FMT material, candidates are admitted as stool donors only after passing a rigorous screening protocol, reviewed by the FDA, that includes clinical, blood, stool, and nasal swab assessments (Supplementary Information) [5, 12]. Prior to March 2020, the stool screening panel included STEC via enzyme immunoassay (EIA). If a donor tested positive for STEC during a screen, they were deferred from the donor program for 8 weeks and their material intended for clinical use but held in quarantine was destroyed.

Screening for STEC was recommended by an international consensus [13], but the consensus did not recommend a specific method for screening. EIA is a method recommended by the US Centers for Disease Control and Prevention for detecting the presence of Shiga toxin in symptomatic patients [14], among whom the assay has a sensitivity of approximately 68% [15]. However, the sensitivity of EIA to detect asymptomatic carriers is unknown. Importantly, screening at the bank before March 2020 did not include a polymerase chain reaction (PCR) test for Shiga toxin production genes (*stx1/2*) nor for STEC virulence factors (*eae* or *ehxA*) [14].

Stool donations are processed into fecal microbiota preparations (FMPs) in a dedicated facility according to phase-appropriate current Good Manufacturing Practice, which includes careful quality manufacturing standards as well as inventory and shipping management. Safety aliquots from every donation are preserved in the case of an adverse event that requires testing of the FMP. All FMPs are assigned an identification number, which allows trace-back to specific donors, dates of donation, and correlation with other donor behaviors and activities. Material tracking records, clinical outcome, and adverse event reports are returned to the stool bank by clinical sites that use the FMPs. The combination of these activities allows the stool bank to detect and investigate adverse events and emerging safety signals.

MATERIAL RECALL AND PATIENT IMPACT

On 18 February 2020, the stool bank received a report of an adverse event occurring in patient A (Table 1, Figure 1, Supplementary Information). On 24 February, safety aliquot testing results from donations from donor X associated with

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Table 1. Adverse Events Reported to the Stool Bank Concerning Patients Treated With FMPs Derived From Donor X

Patient	Age (Years), Sex	Indication for FMT	Comorbidities	Adverse Events	Delay Between FMT and Adverse Event	Patient Stool Testing Post-FMT	Outcome	Relatedness[a]
A	16, F	Moderate rCDI	IBD	Transient abdominal pain	1 day	None performed	Outpatient, resolved without sequalae	Nonserious[b]
B	70, F	Moderate rCDI	Microscopic colitis	Diarrhea	18 days	*Clo*stridioides *difficile* (PCR), STEC (PCR)	Outpatient, resolved without sequalae	Related
C	53, F	Fulminant CDI	Ehlers-Danlos syndrome, Crohn's disease, dysautonomia	Diarrhea, abdominal pain	1 day	EAEC (PCR), STEC (PCR)	Hospitalization, recovered without sequalae	Related
D	87, F	Severe rCDI	CCF, hypertension, dementia, hypothyroidism	Diarrhea	26 days	*C. difficile* (PCR), norovirus (PCR), STEC (PCR)	Hospitalization, recovered without sequalae	Related
E	77, M	Moderate rCDI	CCF, ischemic heart disease, CKD, DM	Decompensated CCF	19 days	None performed	Death	Not related[c]
F	78, F	Recurrent CDI	Unknown	Recurrent diarrhea, sepsis	>30 days	None performed	Death	Not related[d]
G	64, F	Moderate rCDI	CHF, renal failure	Mild diarrhea, CDI recurrence	16 days	*C. difficile* (PCR), STEC (PCR)	Outpatient, recovered without sequalae	Related[e]

Abbreviations: CCF, congestive cardiac failure; CDI, *Clostridioides difficile* infection; CHF, congestive heart failure; CKD, chronic kidney disease; DM, diabetes mellitus; EAEC, enteroaggregative *Escherichia coli*; F, female; FMP, fecal microbiota preparation; FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; M, male; PCR, polymerase chain reaction; rCDI, recurrent CDI; STEC, Shiga toxin–producing *Escherichia coli*.

[a]Relatedness as determined by the stool bank at the end of the investigation.

[b]Nonserious adverse events are not assigned a relatedness.

[c]FMP provided to the patient tested STEC-positive by PCR, but the reporting clinician stated that the patient showed no clinical evidence of STEC, and the patient died due to cardiorenal failure unrelated to FMT.

[d]The long delay between FMT and the adverse event, the patient's clinical course, and the fact that the FMP provided to the patient tested STEC-negative by PCR led to this determination.

[e]Whole-genome sequencing of a patient STEC isolate confirmed transmission via FMT.

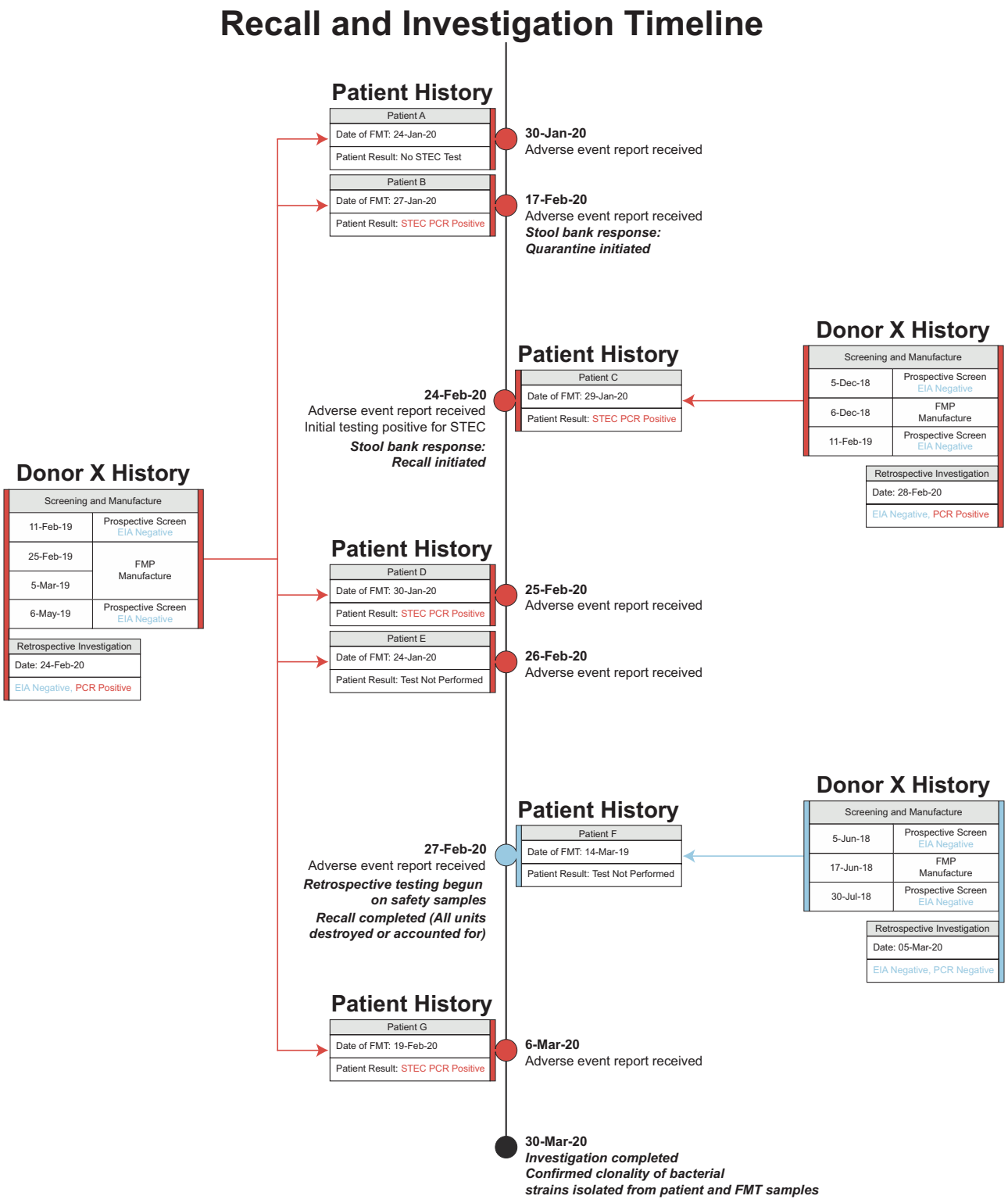

Figure 1. History of donor X's screening and donations relevant to the investigation ("Donor X History"), patient histories, and timeline of recall and investigation. Arrows show what material was used in which patients. EIA testing of bookends was performed as part of standard, prospective screening. PCR tests were conducted as part of the retrospective investigation. Patient F, whose FMT predates those of other patients, was presumably reported because of the bank's 24 February 2020 request for physicians to report adverse events associated with material from donor X. Abbreviations: EIA, enzyme immunoassay; FMP, fecal microbiota preparation; FMT, fecal microbiota transplantation; PCR, polymerase chain reaction; STEC, Shiga toxin–producing *Escherichia coli*.

patient A's adverse event returned positive for STEC by *stx1/2* PCR, and the bank received the second report of a potential STEC transmission event (patient B). The patients had tested positive to STEC on a gastrointestinal multiplex PCR panel for non-O157 STEC and *C. difficile* after undergoing investigation for symptoms consistent with CDI recurrence. Although both treating physicians determined that the STEC finding was incidental and likely unrelated to the patient's CDI recurrence, the bank requires reporting of adverse events and was informed of these events by the treating physicians. Because both patients had been treated with FMPs derived from stool donated by 1 stool donor, donor X, the bank immediately placed all of donor X's FMPs at the bank into quarantine, informed all sites that had received material from donor X, requested that those sites conduct follow-up and to report any serious adverse events to the bank, and initiated a recall of donor X's material. The bank also initiated an investigation, described below, to determine if STEC was indeed transferred via FMT.

Three days after the recall began, the stool bank had successfully confirmed that all unused treatments from this donor had been recalled or destroyed. Of 504 FMPs from donor X, 408 (81%) had already been used in patients. Another 89 (18%) FMPs were confirmed destroyed or no longer in usable inventory. The remaining 7 (1.4%) FMPs were part of investigator-led clinical trials and the principal investigator and study staff were appropriately informed.

Between 30 January and 22 May 2020, a total of 6 serious adverse events and 1 nonserious adverse event were reported from patients who received donor X FMPs (patients A through G) (Table 1, Supplementary Information). Informed by the investigation described below, the bank determined that 4 serious adverse events (patients B, C, D, and G) were related to the FMT, 2 deaths [10] (patients E and F) were not related to the FMT, and the 1 nonserious event resolved without additional testing or follow-up (patient A). Testing performed as part of the investigation showed that the FMP used to treat 1 of the patients who died (patient F) did not contain STEC [11]. The FMP used to treat the second patient who died (patient E) was positive for STEC by PCR, but the reporting clinician stated that the patient had resolution of their CDI diarrhea and showed no clinical evidence of STEC infection, hemorrhagic colitis, or hemolytic uremic syndrome. The patient, who suffered from congestive cardiac failure and chronic kidney disease, died due to cardiorenal failure unrelated to FMT.

INVESTIGATION

In tandem with the material recall, the bank conducted an investigation to determine whether these reports represented a transmission of STEC organisms from donor X via the FMT material. The investigation addressed multiple questions concurrently; the results here are organized by theme rather than chronological order.

To assess whether EIA testing was sufficiently sensitive to detect STEC carriage but the "bookend" screens were not sufficiently frequent to detect carriage, safety aliquots from the implicated donations were tested for STEC by EIA (Figure 1). The tests were negative, suggesting that more frequent EIA testing would not have detected carriage of STEC in this asymptomatic donor.

To evaluate if a nucleic acid amplification test could detect asymptomatic STEC carriage, safety aliquots from the implicated stool donations, as well as other donations from donor X for comprehensiveness, were tested for STEC using *stx1/2* PCR. Of 20 donations tested, 11 donations, including the 3 used to treat patients A, B, C, D, E, and G, tested positive by PCR despite testing negative by EIA. These findings indicated that prospective *stx1/2* PCR testing of the donor may have detected the donor's asymptomatic STEC carriage.

To verify that STEC was transmitted via FMT, isolates were cultured from donor X's 3 implicated donations and compared with an isolate collected from patient G (Table 1, Figure 1). Genomes from the 3 donor X isolates and the 1 available patient isolate were sequenced and compared to assess clonality using the epiXact analysis service (Day Zero Diagnostics, Cambridge, MA), as described previously [8]. In silico analysis showed that all 4 isolates belonged to serotype H7:O117 and that the donor and patient isolates were likely clonal (0–1 single nucleotide polymorphisms [SNPs] between the donor isolates; 0–6 SNPs between donor isolates and patient isolate), confirming transmission of STEC from donor X to patient G. In contrast, an isolate of the same serotype isolated in Germany was 761–770 SNPs distinct from the donor and patient isolates [16]. STEC isolates were not available from any other patient as no samples or isolates had been retained by the clinical sites and no patient required further stool testing as part of their clinical care.

On 10 March 2020, in consultation with the FDA, the stool bank began implementing *stx1/2* PCR testing prospectively for all donor screening. The FDA now recommends that all donor stool used for FMT be tested by nucleic acid amplification tests for STEC [10].

Further results of the investigation are shown in the Supplementary Information.

DISCUSSION

This is the first report of large-scale surveillance detecting transmission of a pathogen via FMT. While the previous report of an infectious transmission was in an FMT program limited to a single healthcare service [8], this report describes surveillance activities covering more than 10 000 FMPs shipped yearly to all 50 US states. This is also the first report of an FMT transmission report in which the organism went undetected despite screening for that organism. In the previous report of

a transmission event, the donor had not been screened for the offending pathogen [8]. To our knowledge, there have been no other transmissions of pathogens via FMT that were confirmed using whole-genome sequencing.

The confirmation of transmission was possible because the investigation was conducted by a large stool bank with surveillance and sufficient quality measures in place to allow for detection and retrospective testing. On its own, a single report of a patient testing positive for STEC on a PCR panel would likely not have led to an investigation capable of confirming the transmission of STEC via FMT and the value of more sensitive PCR testing. Thus, these surveillance capabilities ensured no additional patients were dosed with STEC-positive material.

Confirmation of transmission was possible because linked donor and patient material was available, allowing for genomic comparison of donor and patient STEC isolates. However, material from only 1 patient was available, limiting the scope of the investigation. We therefore strongly encourage clinicians performing FMT to retain patient samples when pathogen transmission via FMT is suspected.

We hope that this model of surveillance, investigation, and rapid implementation of an updated screening protocol will inform standards for all human-derived microbial therapeutics. As with blood banking and other human-derived therapies, methods of detection, adverse event surveillance strategies, and risk-management plans should be appraised and updated regularly. We call for the inclusion of screening test specifications and minimum standards for surveillance in universal screening guidelines [13]. Finally, we note that all human-derived microbial therapeutics, even those distributed under a traditional FDA approval, will need to continually evolve as new safety considerations are detected and ameliorated.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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