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A cluster of Shiga Toxin-producing *Escherichia coli* O157:H7 highlights raw pet food as an emerging potential source of infection in humans

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Abstract

In August 2017, a cluster of four persons infected with genetically related strains of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 was identified. These strains possessed the Shiga toxin (*stx*) subtype *stx2a*, a toxin type known to be associated with severe clinical outcome. One person died after developing haemolytic uraemic syndrome. Interviews with cases revealed that three of the cases had been exposed to dogs fed on a raw meat-based diet (RMBD), specifically tripe. In two cases, the tripe had been purchased from the same supplier. Sampling and microbiological screening of raw pet food was undertaken and indicated the presence of STEC in the products. STEC was isolated from one sample of raw tripe but was different from the strain causing illness in humans. Nevertheless, the detection of STEC in the tripe provided evidence that raw pet food was a potential source of human STEC infection during this outbreak. This adds to the evidence of raw pet food as a risk factor for zoonotic transmission of gastrointestinal pathogens, which is widely accepted for *Salmonella*, *Listeria* and *Campylobacter* spp. Feeding RMBD to companion animals has recently increased in popularity due to the belief that they provide health benefits to animals. Although still rare, an increase in STEC cases reporting exposure to RMBDs was detected in 2017. There has also been an increased frequency of raw pet food incidents in 2017, suggesting an increasing trend in potential risk to humans from raw pet food. Recommendations to reduce the risk of infection included improved awareness of risk and promotion of good hygiene practices among the public when handling raw pet food.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are a group of bacteria associated with human disease and are defined by the presence of one or both phage-encoded Shiga toxin genes: *stx1* and *stx2*. In the UK, STEC serotype O157:H7 is the most common type and around 700 cases of STEC O157:H7 are reported annually in England. Although this is relatively low compared to around 10 000 *Salmonella* and 60 000 *Campylobacter* cases (https://www.gov.uk/government/publications/zoonoses-uk-annual-reports), STEC O157:H7 are of significant public health concern due to the potential severity of the disease. Symptoms can range from mild diarrhoea to include abdominal cramps, vomiting and severe bloody diarrhoea. In 5–15% of cases, infection can lead to the development of haemolytic uraemic syndrome (HUS), a severe multisystem syndrome [1]. The risk of developing HUS following STEC infection varies by age and gender; HUS is most commonly seen in children under 5 and is recognised as the most common cause of acute kidney failure in children in the UK. Although extremely rare, HUS can be fatal, particularly in infants, young children and the elderly. Certain STEC strains have been shown to be more often associated with developing HUS than others, with those possessing *stx2*, particularly the *stx2a* sub toxin type most associated with severe disease [1–3].

STEC are zoonotic and healthy ruminants, particularly cattle and sheep, are the main reservoirs of infection. STEC has a very low infectious dose and transmission to humans occurs through the consumption of contaminated food or water, direct or indirect contact with infected animals or their environment and through person to person spread. Each transmission route can cause sporadic infection as well as outbreaks.

Enhanced monitoring of STEC infections in England is undertaken by Public Health England (PHE). Since 1 January 2009, the National Enhanced STEC Surveillance System has collected detailed epidemiological data on every case of STEC O157:H7 in England [4].

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In addition, isolates of STEC identified at local diagnostic laboratories are referred to the Gastrointestinal Bacteria Reference Unit at PHE for confirmatory testing. Since July 2015, whole genome sequencing (WGS) has been employed on all STEC to provide highly discriminatory typing for public health surveillance and to facilitate outbreak detection and investigation [5–8].

In August 2017, a cluster of four cases infected with genetically related strains of STEC O157:H7 was identified by the national Gastrointestinal Pathogens Unit Department (GIPU) at PHE. The strains possessed the *stx2a*, known to be associated with more severe disease and HUS. Furthermore, one case died after developing HUS. Despite the small numbers of cases, due to the high disease severity, a multi-agency investigation was under taken and the findings are reported here.

Methods

Microbiological investigations

Faecal specimens from General Practitioner and hospital patients were processed in local hospital microbiology laboratories for identification of *Salmonella*, *Campylobacter*, *Shigella* spp. and STEC O157:H7. Presumptive STEC O157:H7 isolates were sent to the PHE Gastrointestinal Bacteria Reference Unit (GBRU) for confirmation and identification of the phage type (PT) and Shiga toxin (*stx*) testing. Further typing was undertaken using WGS, where the bacteria are compared genetically to each other and to STEC O157:H7 isolates previously obtained from humans, animal and food samples across England.

For WGS, DNA was extracted from the cultures of STEC O157:H7 for sequencing on the Illumina HiSeq 2500 instrument. Quality-trimmed Illumina reads were mapped to the STEC O157:H7 reference genome Sakai (GenBank accession BA000007) using BWA-MEM [9, 10]. Single-nucleotide polymorphisms (SNPs) were identified using GATK2 (ref3) in unified genotyper mode [11]. Core genome positions that had a high quality SNP (>90% consensus, minimum depth 10×, GQ ≥ 30) in at least one isolate were extracted for further analysis. Genomes were compared to the sequences held in the PHE STEC O157:H7 WGS database. STEC O157:H7 with <5 SNP differences within their core genome are considered closely related and likely to have an epidemiological link [5, 11].

Epidemiological investigations

The objectives of the epidemiological investigation were to identify and describe cases associated with the outbreak, and to identify and confirm the likely source/vehicle of the outbreak. Key strands of the epidemiological investigation included agreeing the outbreak case definition, case ascertainment, collection and review of epidemiological data for hypothesis generation and confirmation.

Case definitions

Microbiological typing results reported from the PHE GBRU were used for case finding and classification using the following definitions:

Confirmed: a case of STEC O157:H7 reported by GBRU as belonging to the same SNP designation with an onset date on or after 23rd June 2017.

Possible: a case of STEC O157:H7 PT 21/28 confirmed by GBRU and awaiting WGS and SNP typing with no known links to concurrent outbreak investigations of this PT.

Case interviews

Local laboratories report presumptive isolates of STEC directly to Health Protection Teams (HPT) within PHE. Each HPT arranges for an enhanced surveillance questionnaire (ESQ) to be completed either directly or via their Local Authority, Environmental Health Officer to interview the patients in a timely manner as part of routine public health follow-up (ESQ available at https://www.gov.uk/government/publications/vero-cytotoxin-producing-escherichia-coli-questionnaire). The ESQ collects data in the following categories: demographic details; risk status; clinical condition (including progression to HUS); household or other close contact details; exposures including travel, food and water consumption, contact with animals and environmental factors; case classification; outbreak status. Completed questionnaires are forwarded for inclusion in the National Enhanced STEC Surveillance System (NESSS) which is managed by the PHE GID.

Trawling exercise

Confirmed cases or a family member were contacted by phone by PHE HPT's in order to complete trawling questionnaires. The trawling questionnaires were much more in-depth with detailed questions on all food handled and consumed within the exposure period as well as detailed questions on environmental exposures. These questionnaires were completed online in SelectSurvey by the interviewers and a script provided.

Food and environmental sampling

Samples of potential food source/vehicles were obtained and tested at the PHE Food, Water and Environmental (FW&E) laboratories. Food, water and environmental samples were collected by Environmental Health Practitioners (EHPs) from the freezers of two cases, an implicated producer of raw pet feed and a pet food shop, and transported in accordance with the Food Standards Agency Food Law Code of Practice (https://www.food.gov.uk/enforcement/codes-of-practice/food-law-code-of-practice-2015) to PHE FW&E microbiology laboratories at Porton and York in cold boxes at a temperature of between 0 and 8 °C and tested within 24 h of collection. Food and environmental samples were tested using PHE Standard Method M6 (based on EN ISO/TS 13136:2012; https://www.iso.org/standard/53328.html), and Method F17 (based on BS EN ISO 16654:2001; https://www.iso.org/standard/29821.html). Isolates of STEC from food and environmental samples were referred to GBRU for further characterisation and WGS. Local EHPs requested supply chain data on the implicated products from the pet food stores.

Ethical statement

The authors declare that there is no requirement for ethical approval for this submission. This study was undertaken to inform the delivery of patient care and to prevent the spread of infection, defined as usual practice in public health and health protection.

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Results

Microbiological investigations

The isolates of STEC O157:H7 from the four cases were PT21/28 *stx2a*. WGS identified that the isolates from three human cases had an identical SNP profile and one case had an SNP profile that was one SNP different to the outbreak profile. Phylogenetic analyses indicated the strain of STEC in this outbreak clustered most closely with other strains isolated from cases reporting that they had not recently travelled outside the UK or from UK animals, and the source of infection was therefore likely to be of domestic (UK) origin. This indicated transmission to human cases was most likely due to (i) direct contact with UK cattle or their environment, (ii) contact with, or consumption of, contaminated meat or dairy products from UK cattle or sheep or (iii) consumption of produce cultivated in close proximity to a ruminant reservoir in the UK.

Epidemiological investigations

Four confirmed cases were identified. Onset dates ranged from 23 June to 23 July 2017. Two of the confirmed cases were female and two were male. The median age was 6 years (range 6–45 years). All four cases were resident in England and cases were distributed among four PHE centres. No cases were detected in Scotland or Wales. All four cases reported diarrhoea, three of which had bloody diarrhoea accompanied by vomiting. One of the cases was still ill when the ESQ was administered and duration of illness ranged from 4 to 8 days at the time of interview. Three cases were hospitalised and one case developed HUS and subsequently died. The cause of death was listed as HUS and sepsis.

Exposure information

None of the cases reported recent foreign travel (within 7 days of onset of symptoms). No cases reported access to private water supplies, however two cases reported swimming and one case reported other water exposure (e.g. canoeing, fishing, sailing and surfing). Four cases reported contact with dogs, one also with chickens. One case specified feeding their dogs' raw tripe. A variety of food exposures were reported on the ESQ's. Cases reported shopping at various different supermarket chains, the food histories were complex and no clear common exposures were apparent. Two cases reported consuming dairy-free products such as soya yoghurt and goats milk and free from products such as gluten-free bread.

A second case had contact with dog(s) also fed on raw tripe purchased from the same shop as that for the first case who reported handling raw tripe. Furthermore, another case had close contact with a dog, including brushing its teeth with their own toothbrush. This dog was also fed a raw meat-based diet (RMBD).

A second trawling interview was undertaken with each case (or a family member) between the 21 and 24 August 2017 with the aim of refining a hypothesis for investigation. The trawling questionnaires indicated that contact with dogs and consumption of raw carrots were the only exposures common to all four cases. Feeding of raw tripe ($n = 2$) and an RMBD ($n = 1$) was reconfirmed in the interviews. The fourth case had contact with a family member's dog that was not reported as being fed tripe or raw pet food. The family were re-questioned specifically around the dog's diet and confirmed the dog was not fed an RMBD. However, they reported contact with another dog fed on bulk frozen pet food sourced from an online company supplying raw pet food, 4 weeks prior to onset of symptoms.

The putative link to raw pet food in this incident led to a retrospective review of exposures to raw pet food among all STEC cases reported to the NESSS. Primary, symptomatic cases with no reporting of foreign travel were included from 1 January 2013 through to 31 December 2017. Of the 2082 cases included in the analysis, 1124 (54.0%) cases reported exposure to dogs and/or cats. Less than a third of those ($n = 353$, 31.0%) reported handling pet food; this is unsurprising given the predominance of child cases of STEC. Handling raw pet food was reported for just 12 cases (3.4%). However, seven of those were reported in 2017, representing 9.1% of cases with exposure to dogs and/or cats in 2017. Of the 12 cases, nine were infected with STEC O157:H7 and three cases with non-O157 STEC strains (including serogroups O76:H19, O113:H4 and O146:H21).

Food and environmental testing

Tripe sampled from a case's home, minced beef from a case's home and a swab of a preparation bench at Producer B were positive for *stx* by polymerase chain reaction (PCR).

STEC O100:H30 *stx2g* was subsequently isolated from the sample of raw tripe taken from a case's freezer and the swab from the preparation bench. The other samples of tripe, dog food and environmental swabs were all negative for STEC by PCR (Table 1).

Product trace-back

Product trace-back was limited. For the pet shop in the South East, the raw pet food had been supplied by a raw pet food producer, distributor and retailer based in the North East (producer/retailer A). Producer/retailer A supplied the pet food for cases in the North East and was supplied by a separate producer (producer B) from which the sample found to be positive for STEC O100:H30 originated from. Producer B stated that they were sourcing tripe from two suppliers, one based in North East England (supplier A) and another based in Northern Ireland (supplier B). The nature of their processes meant it was not possible to determine which of the two sources the STEC contaminated product came from. They also reported sourcing raw meat prior to the onset of symptoms in the cases linked to the outbreak from a third supplier in the North East (supplier C) which went into administration on 24 July 2017 primarily due to hygiene issues.

Discussion

National surveillance using WGS data enabled the detection and investigation of this small, nationally dispersed cluster. Prior to the introduction of WGS this cluster would have occurred below the surveillance radar due the small size, geographical dispersal of the cases and commonly reported PT (PT21/28), comprising approximately a third of STEC O157:H7 cases in England [4]. The benefits of WGS for national surveillance of STEC have been previously demonstrated [12], and in this case facilitated the identification of a potential novel vehicle for STEC transmission.

Due to the small number of cases, epidemiological investigations were limited but did point to exposure to raw pet food, specifically tripe, as a plausible source in three of the four cases. Although one case was not linked to raw pet food, as cattle and

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Table 1. Food and environmental sampling and results undertaken as part of the investigation

Sample number	Date of submission	Place of sampling	Sample description	Results
1	24/08/2017	Home of case	Tripe	STEC by PCR – presumptive positive in 25 g STEC by culture – Isolated STEC referral result – confirmed as *E. coli* serotype O100:H30; ST 993; *stx2g*; eae gene negative; culture positive for *stx* genes
2	24/08/2017	Home of case	Minced beef	*E. coli* O157 culture –not detected in 25 g STEC by PCR – presumptive positive (toxin VT1 and VT2 positive, eae and *E. coli* O-type O157 positive) STEC by culture – not isolated
3	24/08/2017	Producer B	Raw tripe	STEC by PCR – not detected in 25 g
4	24/08/2017	Producer B	Raw tripe	STEC by PCR – not detected in 25 g
5	24/08/2017	Producer B	Swab of prep bench	STEC by PCR – presumptive detected in swab STEC by culture – isolated STEC referral result – confirmed as *E. coli* serotype O100:H30; ST 993; *stx2g*; eae gene negative; culture positive for stx genes
6	24/08/2017	Producer B	Swab of hook that tripe hangs on	STEC by PCR – not detected in swab
7	24/08/2017	Producer B	Swab of freezer shelf	STEC by PCR – not detected in swab
8	24/08/2017	Producer B	Swab of outside of blue box	STEC by PCR – not detected in swab
9	24/08/2017	Producer B	Outside of water bath	STEC by PCR – not detected in swab
10	24/08/2017	Producer B	Water from bath	STEC by PCR – not detected in 1 litre
11	30/08/2017	Pet food shop supplied by producer A	Dog food (tripe and offal)	STEC by PCR – not detected in 25 g
12	30/08/2017	Pet food shop supplied by producer A	Dog food (tripe and offal)	STEC by PCR – not detected in 25 g
13	30/08/2017	Pet food shop supplied by producer A	Dog food (tripe and offal)	STEC by PCR – not detected in 25 g

sheep are the main reservoir of STEC in the UK, exposure to the same strain of STEC may have occurred through a different route. This may be indirect or direct exposure to the infected animals which entered the pet feed supply chain for example. Alternatively, the case may have been exposed to an animal fed an RMBD without being aware of, or being able to recall that exposure.

STEC O157:H7 is detected in ~20% of farms housing cattle in the UK [13]. Tripe is the edible lining of ruminant's stomachs and as such raw tripe can contain zoonotic pathogens including STEC. Although tripe is cleaned and treated for human consumption, many raw pet foods contain green tripe, a raw product which has not been cleaned and contains the untreated contents of the cow's stomach. Sampling and microbiological screening of raw pet food by PCR in this investigation indicated the presence of STEC in three samples. Subsequently, STEC was isolated from one sample of raw tripe collected from a case's home but was different to the strain causing illness in the humans. STEC of the same serotype and *stx* profile was also detected in a swab of a preparation bench at the producer's premises. The outbreak strain was not recovered from the samples of tripe tested during this investigation. However, there are a number of caveats to interpreting these results; the samples tested were not necessarily the batches fed to the case's pets; the infectious dose of STEC is very low and may be below the limits of the tests and there may be uneven distribution of bacteria in the products which meant sampling could miss an affected part of product. Nevertheless, the detection of STEC in the tripe provided evidence that raw pet food was a potential source of human STEC infection during this outbreak.

It is widely accepted that raw meat, including animal by-products used in pet feeds, can contain pathogens which are harmful to health. A recent microbiological study of commercial RMBD products on sale in the Netherlands found *E. coli* serotype O157:H7 in 23% of tested product, as well as *Listeria monocytogenes* (54%) and *Salmonella* species (20%) [14]. Raw pet foods have the potential to cause human disease if contaminated products are consumed, handled or via secondary transfer from contact with contaminated surfaces, for example, kitchen surfaces or dog bowls.

There is evidence of *Salmonella*, *Listeria* and *Campylobacter* being carried by clinically healthy companion animals [15–20]. RMBDs are a reported risk factor for faecal carriage of salmonella, and certain *E. coli* by companion animals [21, 22]. These data suggest therefore that companion animals fed RMBD's may present a zoonotic risk for human infection.

Feeding raw meat to companion animals has recently increased in popularity due to both improved availability and more widespread belief that they provide health benefits to animals. Although still rare, an increase in STEC cases reporting exposure to raw meat was detected in NESSS in 2017. The Animal & Plant Health Agency (APHA) in the UK, responsible

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for approval and monitoring of raw pet food producers, reported an increase in manufacturers from 5 in 2013, to 90 (with 23 awaiting approval) by February 2018 [23]. The FSA reported data on the frequency of raw pet food incidents (microbiological contamination events) in 2017 including data on imports into the UK and exports from UK producers: 10 incidents were reported, 8 were microbiological. Together these data suggest an upward trend in potential risk to humans from raw pet food.

The Pet Food Manufacturing Association (PFMA) Raw Pet Food Group developed, in conjunction with Department for Environment Food & Rural Affairs (Defra), the APHA, PHE and FSA published the Guidelines for the Manufacture of Raw Pet Food on 20 September 2017 (https://www.pfma.org.uk/_assets/docs/raw/Raw-Pet-Food-Guidelines-Oct-17.pdf). These guidelines are intended to improve safety, hygiene and nutrition of raw pet food made in the UK. Meanwhile, there are legislative requirements for regular microbiological testing for *Salmonella* and *Enterobacteriaceae*, where findings demonstrate microbial levels above those stipulated in the regulation, rapid action is taken to address this non-compliance including recall of product where appropriate [23]. Testing, however, is not required for *Listeria*, *Campylobacter* or STEC. Recommendations to reduce the risk of infection included improved awareness of risk and promotion of good hygiene practices among the public when handling raw pet food.

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