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A Foodborne Outbreak of Cryptosporidiosis Likely Linked to Salad Leaves

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Abstract

Aims
We describe an outbreak of cryptosporidiosis and the outbreak control team response.

Methods
An outbreak control team was convened to investigate a cluster of cryptosporidiosis cases notified from a single South Dublin laboratory in July 2020. All cases were interviewed, and Environmental Health Officers conducted 110 food premises inspections to collect food and water samples.

Results
Forty cases were linked to the outbreak, of which 33 fulfilled the confirmed case definition. Thirty-one (78%) of cases were aged 20 to 40 years old. Fourteen (35%) cases required hospitalisation. Several cases shared a common restaurant history. Cross-referencing of food exposures identified a common salad box served in the implicated restaurants, sourced from a single farm, which lead to a precautionary product recall. Sample genotyping of 13 isolates identified *C. parvum*, gp60 subtype IIaA18G3R1 as the outbreak strain. Testing of water and salad leaf samples failed to detect *Cryptosporidium* oocysts.

Conclusion
This investigation highlights the surveillance value of routine PCR screening of stool samples for *Cryptosporidium*, which facilitated early detection of this outbreak. However routine surveillance remains inconsistent at Irish and European level. To accurately measure the incidence of *Cryptosporidium* infection in Europe, a comprehensive, uniform surveillance system is needed.

Keywords: Surveillance, cryptosporidiosis, Cryptosporidium, Ireland, foodborne, outbreak, food safety, salad

Introduction

Cryptosporidium is a protozoal parasite which causes the diarrhoeal disease cryptosporidiosis. *Cryptosporidium* species can be found in water, soil, food or any surface which has been contaminated with human or animal faeces.

Primary transmission occurs via the faeco-oral route from either animals or humans. Secondary, person to person, transmission can also be a feature of outbreaks particularly in the case of food handlers or caregivers. *Cryptosporidium* oocysts have the potential to cause large outbreaks as they are highly resistant to chlorine based disinfectant and can survive many months in the environment [1, 2]. Contamination of public water supplies [3, 4] and fresh produce [5-9] have been implicated in a number of large European outbreaks of cryptosporidiosis.

Clinical presentations of infection can range from mild GI upset to severe diarrhoea necessitating hospital admission. The incubation period is usually 5 – 7 days although wider ranges have been reported [10, 11]. There is no specific therapy licensed in the EU and treatment is limited to supportive care.

Cryptosporidiosis is a notifiable disease in Ireland. Previous reported outbreaks have been related to contaminated water supplies[12]. High yearly rainfall and open reservoirs likely contribute to the vulnerability of Irish water system to contamination [13]. In 2019, a total of 91 cases of cryptosporidiosis were notified to the Public Health Department of the Greater Dublin area; a background notification rate in the order of zero to five cases per week. Since 2012 Ireland has consistently reported the highest annual rate of cryptosporidiosis in the European Union (EU)[14].

Methods

On the 28th July 2020, a cluster of nine cases of *Cryptosporidium* were notified to the local Public Health Department by a single laboratory in the South Dublin/Wicklow area. An outbreak was declared and an outbreak control team (OCT) convened the same day. All notified cases were contacted by phone to discuss enteric precautions and complete a standardised exposure questionnaire. Questionnaires focused on potential high-risk exposures, such as salad leaves, unpasteurised dairy, private water supply or contact with animals. Information was collated using Microsoft Excel and analysis was performed using SAS (University version).

A likely foodborne source was suspected from the preliminary outbreak investigation, based on the frequency of reported restaurant exposures, and an initial cluster of six cases involving a single restaurant. The following case definitions were then used by the OCT to classify cases in the outbreak:

Confirmed: laboratory confirmed cryptosporidiosis with onset of gastrointestinal symptoms from July 16th, 2020 onwards, who ate in a common food premises in the 14 days prior to symptom onset.

Possible: laboratory confirmed cryptosporidiosis with onset of gastrointestinal symptoms from July 16th, 2020 onwards, who ate in any food premises in the Dublin/Wicklow area in the 14 days prior to symptom onset.

Sporadic: laboratory cases of cryptosporidiosis notified from July 16th onwards without any history of having eaten in a food premises in the Dublin/Wicklow area in the 14 days prior to symptom onset.

Environmental Health Officers began inspections of food businesses in which suspected cases had consumed food in the 14 days preceding the onset of their symptoms. In total, 110 inspections of food businesses and suppliers were carried out.

An extensive cross-check of restaurants, suppliers and salad products was undertaken. One particular product, a seasonal salad box, was identified as having been supplied to all premises associated with these 31 cases. Two further cases (household contacts) were epidemiologically linked to the outbreak. The salad box was produced by Farm A and shipped exclusively by two suppliers. Five 25g samples of salad leaves were collected from these suppliers on 29th, 30th July and 4th August 2020 and tested for presence of cryptosporidium oocysts by Eurofins Biomnis Laboratory. The customer lists of the suppliers were then obtained to identify other premises to which the product had been distributed.

Farm A primarily produced baby leaf salad but was a mixed enterprise with livestock in adjoining fields. There was a private well onsite at the farm; from which a 10 litre water sample was analysed for *E. coli*, *Enterococci* and *Cryptosporidium*. The OCT was informed that the well water was not used for washing of the salad leaves but rather for use in steam cleaning of the sorting bench and product containers and crates after production.

Cases were laboratory confirmed as detection of *Cryptosporidium* species by real time PCR testing using EntericBio Gastro Panel II (SeroSep). Sixteen stool specimens from the outbreak were sent to Cryptosporidium Reference Unit, Wales.

Results

Epidemiological Results

A total of 54 laboratory confirmed *cryptosporidium* cases were notified from 23rd July to 6th August. Of these cases, 40 were part of the outbreak as specified by the outbreak case definition (33 were classified as confirmed cases and 7 as possible cases).

Of the confirmed cases, two were likely secondary transmission as household contacts of a case. Two cases diagnosed outside of Dublin had both recently eaten in an implicated food premises in Dublin.

Dates of symptom onset ranged from 16th to 29th July with a peak of 12 cases on 23rd July (figure 1). Table 1 displays the descriptive analysis of the 40 cases linked to the outbreak. Thirty one cases (76%) were aged 20 – 40 years old. The minimum age was 3 years and the maximum age was 74 years. Thirty one cases (76%) were resided in the South Dublin and North Wicklow area. Fourteen cases (35%) required inpatient hospital treatment.

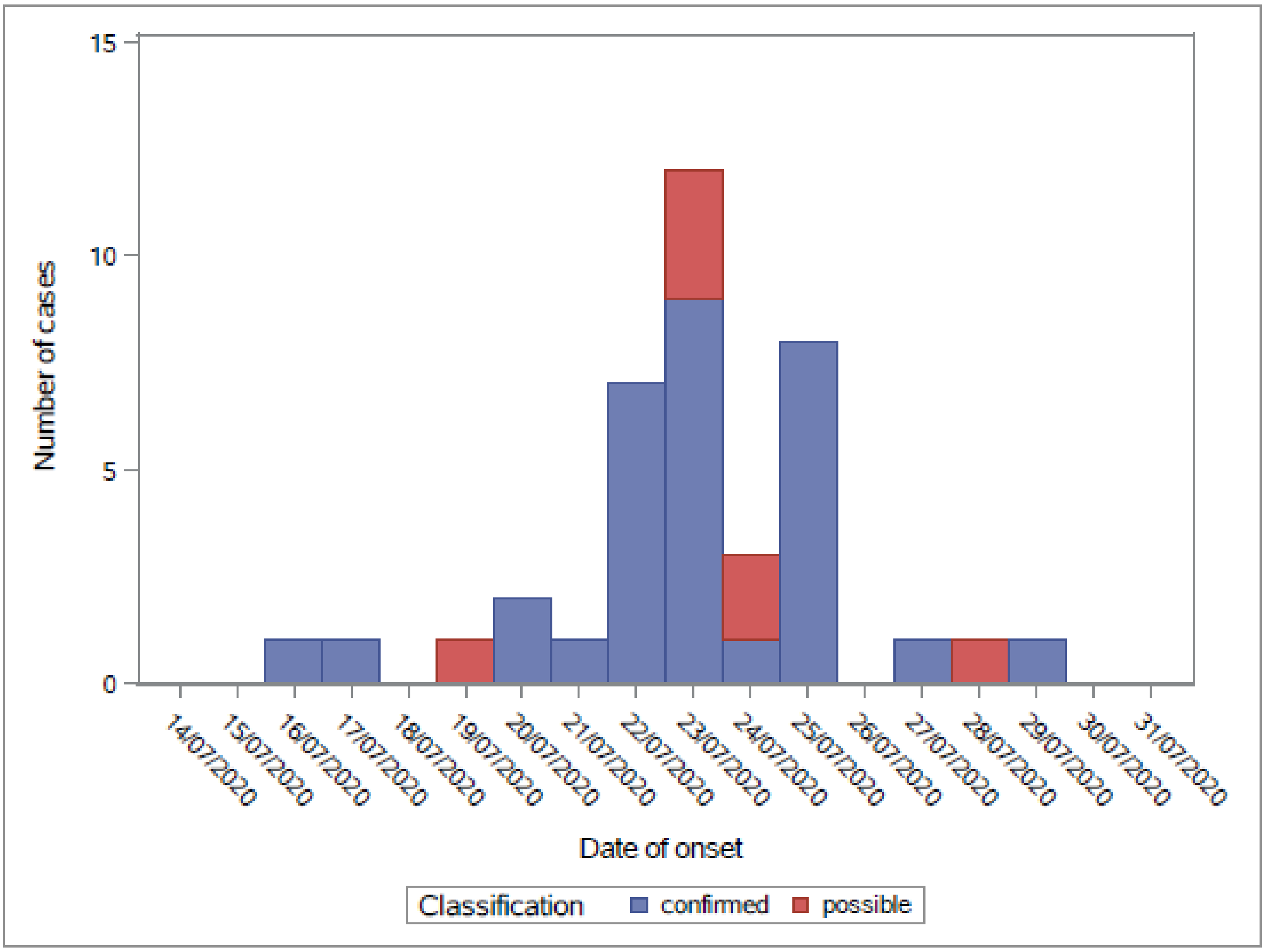

Figure 1: *Epidemic curve by date of onset for 40 cases of cryptosporidiosis linked to the foodborne outbreak in the Greater Dublin area in July 2020.*

Table 1: *Descriptive analysis of 40 cases and isolates of cryptosporidium linked to the foodborne outbreak in the Greater Dublin area in July 2020.*

	Confirmed (n=33)	%	Probable (n=7)	%	Total (n=40)
Sex					
Female	21	(63.6)	4	(57.1)	25
Male	12	(36.4)	3	(42.9)	15
Age group					
1-19	4	(12.1)	1	(14.3)	5
20-29	17	(51.5)	1	(14.3)	18
30-39	9	(27.3)	4	(57.1)	13
40-49	1	(3.0)	1	(14.3)	2
50-59	1	(3.0)	0	(0.0)	1
>60	1	(3.0)	0	(0.0)	1
Public Health Area					
South Dublin/Wicklow	28	(84.9)	3	(42.9)	31
West Dublin/Kildare	4	(12.1)	2	(28.6)	6
North Dublin	0	(0.0)	1	(14.3)	1
Other	1	(3.0)	1	(0.0)	2
Patient diagnosis					
Community	14	(42.4)	5	(71.4)	19
Emergency Department	6	(18.2)	1	(14.3)	7
Hospital inpatient	13	(39.4)	1	(14.3)	14
Genotype					
IIaA18G3R1	11	(84.6)	1	(33.3)	12
PCR negative	1	(7.7)	1	(33.3)	2
Un-typable	1	(7.7)	1	(33.3)	2
Not typed					24
Swimming					
No	16	(76.2)	1	(33.3)	17
Yes	5	(23.8)	2	(66.7)	7
Unknown					16
Travel					
No	17	(77.3)	3	(100.0)	20
Yes	5	(22.7)	0	(0.0)	5
Unknown					15

Given the geographic clustering of cases, a survey of all public and private hospital laboratories in five surrounding public health areas was conducted to establish whether testing for *Cryptosporidium* was part of routine stool sampling protocol (Table 2). Of the 18 laboratories surveyed: 8 (44%) screened routinely using PCR method, while 10 (56%) tested only if specifically requested.

Table 2: *Cryptosporidium testing protocol in 18 hospital laboratories across 5 public health areas.*

Public Health Area	Hospital laboratory	Cryptosporidium testing protocol of stool samples
South Dublin/Wicklow	A	Routine PCR
	B	On request
	C	On request
West Dublin/Kildare	D	On request
	E	On request
	F	On request
	G	Routine PCR
	H	Routine PCR
	I	Routine PCR
North Dublin	J	On request
	K	On request
	L	On request
	M	Routine PCR
South-East	N	Routine PCR
	O	Routine PCR
North-East	P	On request
	Q	On request
	R	Routine PCR

Environmental Results

All inspected food premises were supplied with water by the public mains system. Ongoing monitoring by the utility company recorded no detection of *Cryptosporidium* and there had been no reported breaches at water treatment plants supplying the area.

A large number of food premises were implicated by food history questionnaire. Five food premises (A, B, D, M and R) were linked to at least three cases each, with symptom onset clustered from 20th July to 25th July (figure 2). Of the 40 total cases, 31 ate at restaurants that sourced salad from a common supplier farm.

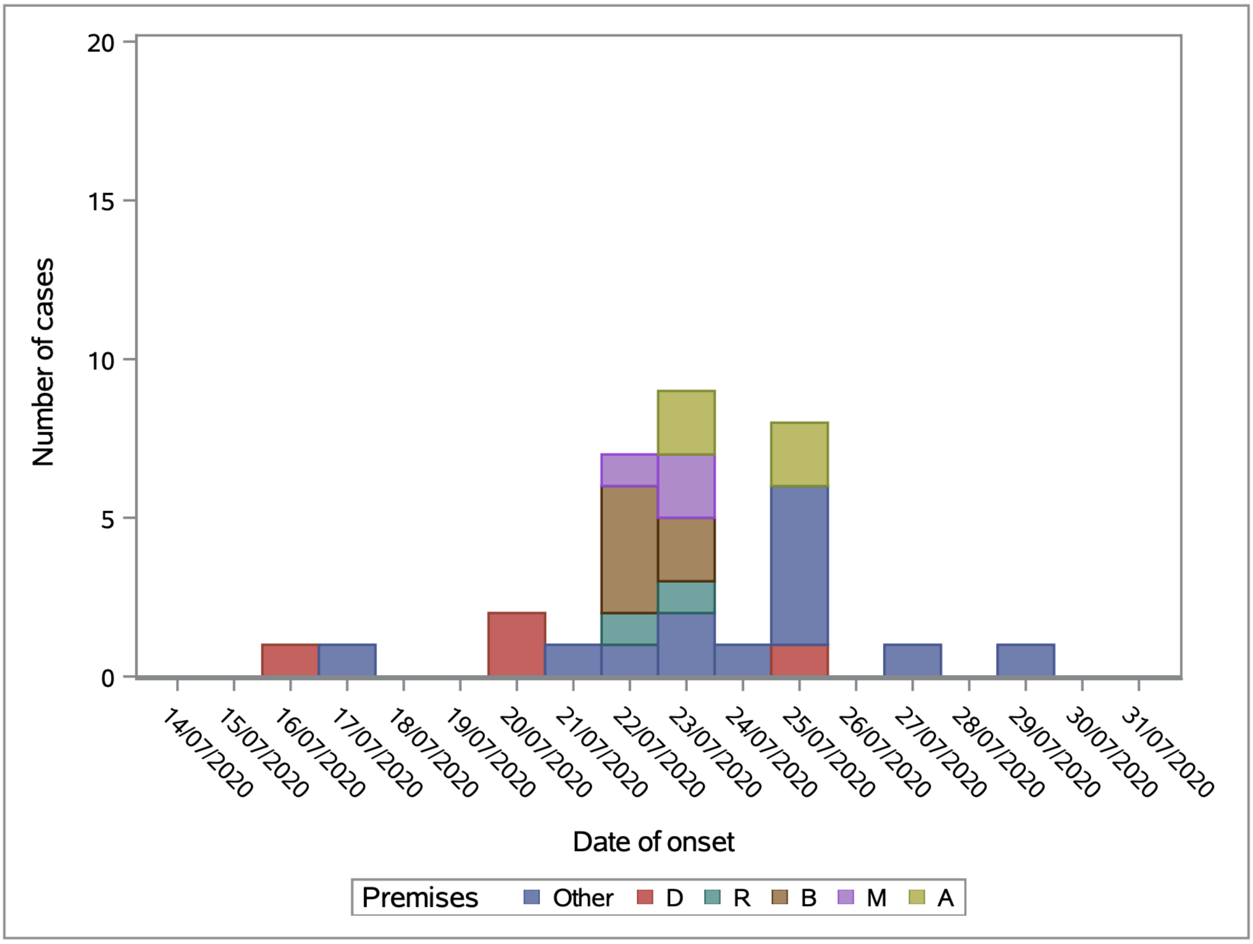

Figure 2: *Epidemic curve of the 33 confirmed cases of cryptosporidiosis linked other the foodborne outbreak by food premises in which the case ate in July 2020.*

The farm owner reported no irrigation of the crops since early June due to adequate rainfall. The crop field did not receive any slurry, straw or organic manure pre- or post-cultivation and was not at risk of flooding and there were no obvious sources of contamination on inspection.

It was noted that a well head on the farm was not adequately protected at the surface or lined to prevent ingress of surface water run-off or shallow groundwater, leaving it vulnerable to contamination. Water testing results from the well were negative for *Cryptosporidium*, *E. coli* and *Enterococci*.

Data from the weather station close to the farm showed that there had been a number of heavy days of rainfall in June following minimal rainfall in May. The largest single day of rainfall measured 20.1 millimetres and occurred on the 20th June. This was within the growing period (28 – 35 days) of the later implicated salad leaves.

Microbiology Results

Sixteen stool specimens from the outbreak were sent to Cryptosporidium Reference Unit, UK, from which 14 PCR positive isolates were identified as *C. parvum* species. Subsequent gp60 genotyping identified subtype IIaA18G3R1 among 12 of the 14 isolates. The remaining two samples could not be typed.

Testing of salad samples did not detect *Cryptosporidium* oocysts or *Giardia*. Two batches of salad were also collected from the only other customer of the farm. Testing of these samples failed to detect *Cryptosporidium* oocysts.

Discussion

The OCT instituted a number of control measures to mitigate the effects of contamination, trace the source of infection and reduce the risk of similar future outbreaks.

Surveillance alerts were issued to regional Public Health Departments, local GPs, hospital microbiologists and Emergency Departments on 29th July. Recommendations issued to Farm A included: fencing off the salad crop field; remediation work to the well head at Farm A, installation of UV disinfection equipment, and washing of salad produce by food premises before use. A product re-call of the seasonal salad box was issued on 4th August as a precaution.

Clustering of cases in place and time suggested a point source outbreak. Although initially focusing on water-based exposures the OCT quickly refocused to food as the potential source of infection. The predominant age range of our cases (20 to 40 years) likely reflected restaurant dining habits as the point of contamination, and the advice given to those over 70 years of age to restrict movements in effect at the time due to the COVID-19 pandemic.

Cryptosporidium parvum is present in a wide variety of animals, particularly sheep and cattle, while IIaA18G3R1 is the predominant gp60 subtype found in Ireland [13, 15]. This was consistent with samples genotyped in this outbreak but, unfortunately, could not further localise the source of infection. Given the three day shelf life of the salad box and the symptom onset date of cases, negative food sample testing cannot rule out contamination of previous salad batches which were not captured in these samples. Ultimately, no microbiological link between the cases and Farm A was established.

Contamination of produce can lead to large foodborne outbreaks. However, cases of cryptosporidiosis are likely under-reported for multiple reasons. Patients may not attend their doctor for testing due to poor awareness of *Cryptosporidium* and the self-limiting nature of symptoms in most cases. Routine laboratory surveillance of *Cryptosporidium* is not robust in Ireland (Table 2). Detection is therefore dependent on awareness among doctors to specifically request *Cryptosporidium* testing. There is a similar lack of harmonisation at a European level [6].

Ultimately, this investigation highlights the surveillance value of routine PCR screening of stool samples for *Cryptosporidium*, which facilitated early detection and management of this outbreak. In order to accurately measure the incidence of *Cryptosporidium* infection in Europe, a comprehensive, uniform surveillance system is needed.

Declaration of Conflicts of Interest:
The authors have no conflicts of interest to declare.

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