UK Health Security Agency

Investigation into acute hepatitis of unknown aetiology in children in England (2022): exposures from food, drink, and water sources

Final report of the IMT, 5 October 2022

Contents

1. Executive summary

This report summarises the stand-alone findings of the environmental exposures investigation undertaken by UK Health Security Agency (UKHSA) scientists up to 29 July 2022, with analysis of the data undertaken up to autumn 2022. It should be read in conjunction with the findings of the technical briefings published by the Agency and the virological findings in those reports.

On 6 April 2022, UKHSA identified an increase in the number of cases of acute hepatitis – without detection of hepatitis viruses (A-E) – in children from England, Scotland and Wales. Since then, further investigations took place and more cases in both the UK and other countries including in Europe were identified. Between 1 January and 4 July 2022, 274 cases of acute non-A to E hepatitis with serum transaminases greater than 500 IU/L in children with no known travel association were identified. Of these 225 (82.1%) of cases were hospitalised and 15 (5.5%) required liver transplantation. The investigation of environmental exposures covered in this report was undertaken up to 29 July 2022, with analysis of the data undertaken up to autumn 2022.

Adenovirus (of all subtypes) was detected in 170 (65.9%) of the 258 cases tested for adenovirus. In a frequency matched case-control study cases were found to have a statistically significant higher odds of concomitant adenovirus infection compared to controls (adjust odds ratio (OR) 35.27, 95% CI 15.23 to 81.68). Amongst a small sample of blood and livers with metagenomics there was also a strong association with adeno-associated virus 2 (AAV2).

Whilst these findings will help guide further investigations into the causes of the outbreak, there is a lack of an apparent direct toxic effect of the virus on the liver and therefore this may be part of a multi-step process. This report focuses on the possibility of an environmental exposure contributing to a multi-step process; it does not consider the plausibility or contribution of other factors in a multi-step process.

The literature review identified that there was a plausible biological pathway for pesticides or mycotoxins to cause or contribute (alongside adenovirus) to hepatitis in children. Duration of this outbreak is potentially less coherent with this hypothesis as crop and trade patterns of potential food, drink or environmental based exposures would limit exposures. However, the international distribution of cases points towards a potential contributory environmental exposure and this warrants further investigation.

Cases' exposures were captured in either a trawling questionnaire conducted on cases up to 28 April, or in a 28-day follow up questionnaire conducted between 7 June and 15 July 2022, or in an in-depth exploratory interview conducted by the Gastrointestinal Infections and Food Safety (One Health) Division in June 2022. The trawling questionnaire asked about exposures in the 7 days before onset of illness since 1 January 2022; the 28-day questionnaire about exposures in

the 2 months before onset of illness; and the exploratory interviews asked about exposures in the 3 months before onset of illness.

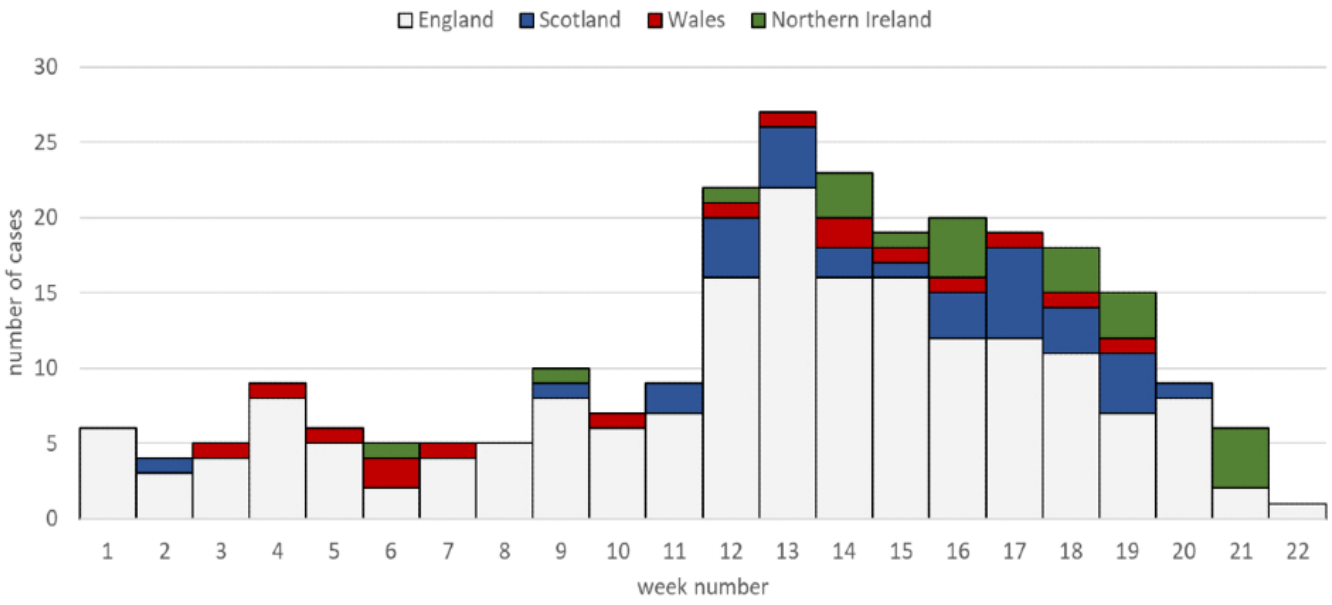
Descriptive analysis of reported case exposures from questionnaires and exploratory interviews identified common consumption of some types of vegetables, fruit, salad, fish, meat and grains of over 20% in questionnaires and over 50% in exploratory interviews. Consumption of these items was compared to a population level dietary survey and a control panel completed for another gastrointestinal outbreak in a similar, but not entirely comparable, population. Based on these crude comparisons we found that reported consumption of some food items, including cucumbers, tomatoes, strawberries, sweetcorn fish fingers and tortilla wraps may have been higher than would be expected, but no single item of food stood out as being consumed by most cases.

Cases continued to be identified after 4 July 2022 but the number of cases declined and the enhanced response to the incident subsequently stepped down. Nevertheless, if case numbers increase in the future, consumption of the food items identified above should be explored further in future questionnaires and, where possible, environmental samples taken, especially since UKHSA toxicology laboratories now have the capability to test for mycotoxins. Such additional data would be required in order to inform further investigations to test the hypothesis of a contributory environmental exposure. Broader recommendations include the use of existing UKHSA guidance and laboratory capabilities for the investigation of clusters of unknown cause; the inclusion of environmental (and non-infectious) exposures and expertise in analytical study design and analysis; and inclusion of such exposures in surveillance schemes collating information on cases over several years.

2. Background

On 6 April 2022, the UK Health Security Agency (UKHSA) identified an increase in the number of cases of acute hepatitis without detection of hepatitis viruses (A-E) in children from England, Scotland and Wales. Since then, further investigations took place and more cases in both the United Kingdom and other countries including in Europe were identified. Between 1 January and 4 July 2022, 274 cases of acute non-A to E hepatitis with serum transaminases greater than 500 IU/L in children with no known travel association were identified (Figure 1). Of these 225 (82.1%) of cases were hospitalised and clinical severity is high with 15 (5.5%) cases who required liver transplantation (1).

Figure 1. Cases of hepatitis of unknown origin by week of presentation, by UK country, 1 January to 13 June 2022 (2)



The clinical syndrome comprises severe acute hepatitis with markedly elevated transaminases (>500 IU/l), sometimes preceded by gastrointestinal symptoms, in children up to the age of 15 years. Jaundice is a prominent feature. The case definitions used are shown below:

Confirmed

A person presenting since 1 January 2022 with an acute hepatitis which is not due to hepatitis A-E viruses, or an expected presentation of metabolic, inherited or genetic, congenital or mechanical cause with serum transaminase greater than 500 IU/L (Aspartate Transaminase AST or Alanine Transaminase ALT), who is 10 years old and under.

Possible

A person presenting with an acute hepatitis since 1 January 2022 with an acute hepatitis which is not due to hepatitis A-E viruses or an expected presentation of metabolic, inherited or

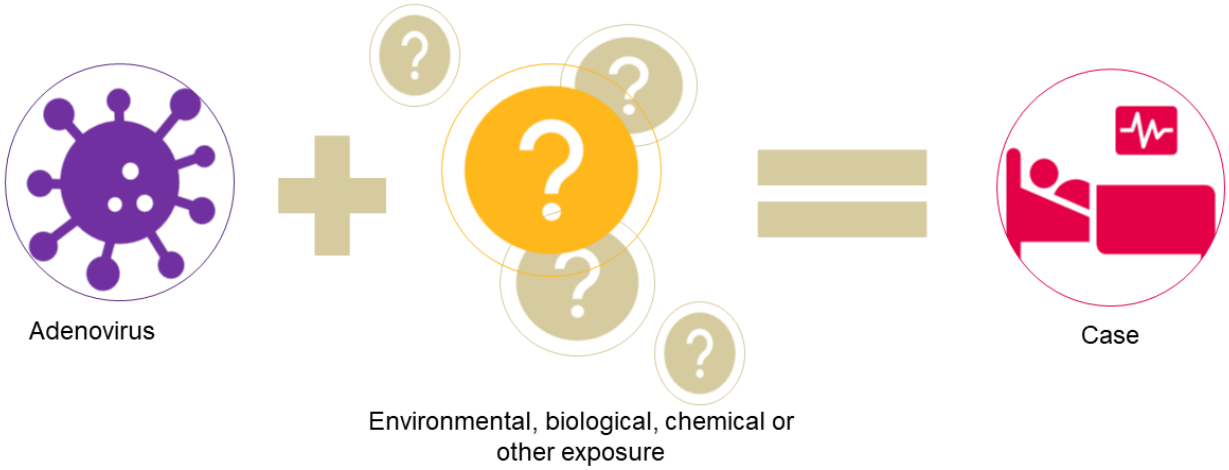
genetic, congenital or mechanical cause with serum transaminase greater than 500 IU/L (AST or ALT), who is 11 to 15 years old.

As at the 28 July 2022, adenovirus (of all subtypes) had been detected in 170 (65.9%) of the 258 cases tested for adenovirus. In a frequency matched case-control study cases were found to have a statistically significant higher odds of concomitant adenovirus infection compared to controls (adjust odds ratio (OR) 35.27, 95% CI 15.23 to 81.68). Amongst a small sample of blood and livers with metagenomics there was also a strong association with adeno-associated virus 2 (AAV2) (4). Whilst these findings help guide further investigations, there is a lack of an apparent direct toxic effect of the virus on the liver and therefore this may be part of a multi-step process (1).

It should also be noted that between 1 November 2021 and 30 April 2022 rates of all subtype adenovirus were higher in those aged 1 to 4 years than at any point in the previous 5 years (1). This rise in adenovirus cases preceded hepatitis of unknown origin cases being identified from January 2022. It also subsided in April, whilst cases of hepatitis continued to be seen. Due to this, and the lack of apparent direct toxic effects of the virus on the liver, other hypotheses have been investigated, including that a multi-step process might be involved whereby "[e]xposure to environmental toxins (for example, mycotoxins found in food), other viruses, and genetic factors [might be] 'triggers' for this phenomenon" (1).

Toxicologists and environmental epidemiologists from the UKHSA's Radiation, Chemical and Environmental Hazards (RCE) directorate worked together with UKHSA virologists, the Agency's Gastrointestinal Infections, Food Safety and One Health Division, and with the Food Standards Agency (FSA), to explore an alternate hypothesis related to an environmental exposure (Figure 2).

Figure 2. Infographic of alternate contributory exposure hypothesis



In May 2022, the collaborative team described above developed an environmental contaminant hypothesis summary which was incorporated into a wider working review of the hypothesis (5, 6). One of the findings of this summary was that the geographical distribution of cases would

support the hypothesis that these cases may be associated with a food or environment contaminant (3, 5, 6). Literature reviews suggest that this could be a pesticide or mycotoxin (as described in Appendix 1). Toxic hepatitis is an inflammation of the liver with can be caused by a reaction to chemical substances, including those found in some pesticides. Toxic hepatitis can develop within hours or may take months to appear. Removing exposure to the toxin can reverse effects, but in some cases toxic hepatitis can permanently damage the liver causing irreversible scarring and liver failure (7).

Mycotoxins are toxic substances produced by a fungus which can be found on a variety of food products as a result of contamination during processing, packaging or storage. The symptoms seen in current cases are similar to those seen in mycotoxin and aflatoxin poisoning (aflatoxins are a type of mycotoxin produced by 2 main species of *Aspergillus*, a type of fungi frequently found in warm climates and on crops including maize, peanuts, cottonseed and tree-nuts) (8). These symptoms are an acute hepatitis with elevated liver function tests which is preceded by gastrointestinal symptoms. Acute exposure to aflatoxins causes aflatoxicosis with fatty infiltration and necrosis of the liver. Reports of outbreaks of ‘acute’ aflatoxicosis in the literature refer to ingestion of contaminated food over a period of weeks or even months (9). Aflatoxicosis presents with varying symptoms including vomiting, abdominal pain, anorexia, malaise, low-grade fever, pulmonary oedema and acute liver failure. There is little information on the risks associated with single acute exposure, although thought to be rare. Aflatoxin poisoning is difficult to diagnose early in humans due to variable symptoms (10, 11). Chronic moderate to high dose exposure can cause abdominal discomfort and swelling, malaise, vomiting, diarrhoea, pyrexia, jaundice and hepatitis. Jaundice and hepatitis may be the presenting features in some cases (10).

The investigation into possible common environmental exposures described in this report started prior to the findings of the strength of association between adenovirus and hepatitis cases being described (4). Initially, the investigation focussed on both potential causative and contributory exposures; the focus shifted later to those factors which may have a potential contributory element to a multi-step process with adenovirus (1).

3. Objectives of this report

3.1 Research question

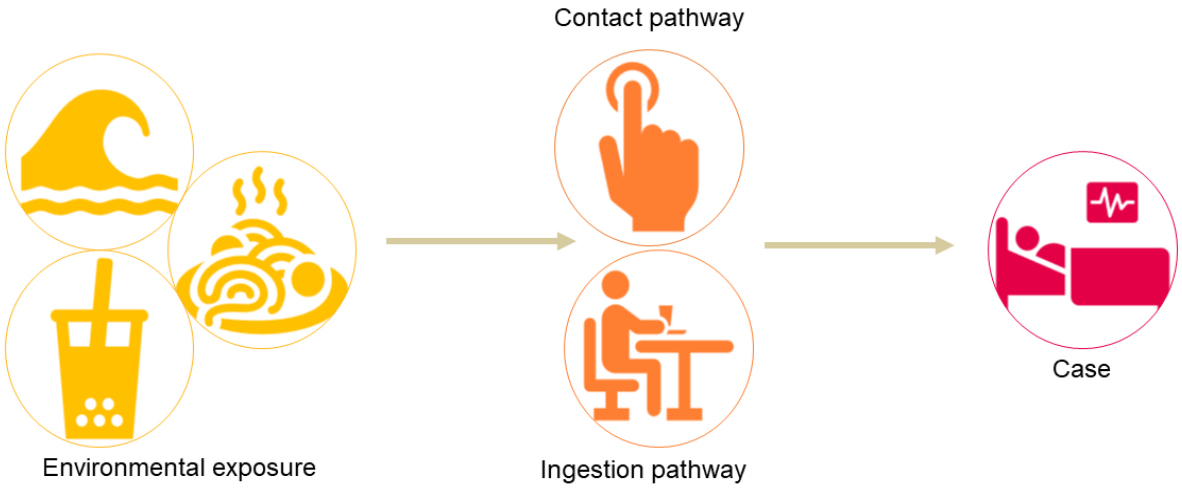
To explore potential food, drink and water-based environmental exposures that could have an association with cases.

3.2 Study objectives

1. Identify: to identify if there are plausible biological pathways (Figure 3) for environmental exposures to cause or contribute (alongside adenovirus) to hepatitis cases in children by:

- undertaking a literature review
- undertaking analytical chemistry
- seeking clinical expert opinion on potential causes of the clinical syndrome

Figure 3. Infographic of environmental exposure pathways



2. Describe: to describe possible food, drink and water exposures for cases from any of the 4 data sources identified as available to the team:

- information contained within the line list up until 19 July 2022
- trawling questionnaires conducted with cases guardians' up to 28 April
- 28-day follow-up questionnaires conducted with cases' guardians between 7 June and 15 July 2022
- in-depth exploratory interviews conducted with 9 cases' guardians by the Gastrointestinal Infections and Food Safety (One Health) Division undertaken during June 2022

4. Methods

This report presents the multi-step methods used in exploring this hypothesis, including literature reviews, clinical opinion, analytical chemistry, descriptive and analytical epidemiology. The literature review, questionnaire and comparative analysis processes are summarised below in a simplified format.

This version of the report has been adapted for publication on the GOV.UK website, meaning that some detailed results have been restricted to protect small numbers and preclude identification of individual responses.

Components (in summary) of the literature review, questionnaire and comparative analysis methodology used to explore this hypothesis

Literature review

To identify potential causative or contributory environmental exposures.

Questionnaires and exploratory interviews to identify exposures exceeding a pre-determined threshold

These comprise a trawling questionnaire, a 28-day questionnaire and exploratory interviews.

- questionnaire reported consumption greater than 20%
- exploratory interview reported consumption greater than 50%

Comparators

- National Dietary and Nutrition Survey
- Market Research Panel for controls from other GI outbreaks

Comparison

- confidence intervals for case consumption and market research panel (MRP) consumption rate calculated
- test of proportions where case consumption exceeded MRP consumption rate

4.1 Literature review methods

4.1.1 Toxicological literature review

Peer-reviewed and grey-literature searches were carried out by the toxicology team to identify potential aflatoxins and chemical agents that could cause hepatotoxicity and those toxins associated with previous outbreaks of aflatoxicosis. Results of the chemicals identified are included in the search terms for adenovirus, mycotoxin and other contaminant terms in the epidemiological search strategy. Results of those toxins associated with previous aflatoxicosis outbreaks are presented by country and year, magnitude of outbreak, clinical syndrome and liver histopathology, and the source and duration of exposure.

4.1.2 Epidemiological literature review

In addition to the toxicological literature search, a rapid literature review was undertaken by UKHSA environmental epidemiology and library services teams. The search questions were developed to review the epidemiological literature on the joint effects of adenovirus and mycotoxins, on liver functions in children, and the joint effect of mycotoxins with other contaminants or viruses and adenovirus with other contaminants or viruses, on liver function in children. This led to the development of 4 key search questions for the literature review, shown in Box 1 below.

Box 1. Search questions for literature reviews

Search 1: Joint Effect of Adenovirus and Mycotoxins

Search 2: Effect of Mycotoxins alone on liver function (hepatitis)

Search 3: Joint effect of Mycotoxins with other contaminants or viruses

Search 4: Joint effect of Adenovirus and any other contaminants or viruses

Literature search strategies were developed based on English language only papers and the inclusion and exclusion concept terms, shown in Box 2 below. (The full search strategy terms used are shown in Appendix 1.)

Box 2. Search concepts for inclusion and exclusion

Inclusion concept terms:

- hepatitis and liver terms
- child terms
- adenovirus and mycotoxin terms
- other viruses and contaminants

Exclusion concept terms:

- Cancer, fungus, mushroom and transplantation terms

We searched 3 databases (Embase, Medline and Scopus) in May 2022. Additional papers of potential relevance were presented by experts and included in the literature review results (but identified as additional papers). Titles of results were screened for relevance by at least 2 members of the team. Abstracts were then reviewed by 2 members of the team for relevance and inclusion and then described by theme.

4.2 Analytical chemistry

4.2.1 Analytical chemistry

Toxicology received plasma or serum, whole blood and urine samples from cases and age-matched controls. The following analyses were undertaken on these samples:

- elemental analysis of whole blood and urine by inductively coupled plasma mass spectrometry (ICPMS)
- untargeted analysis of chemical components of plasma/serum by liquid chromatography-high resolution orbitrap mass spectrometry (LC-HRMS) coupled to bioinformatics analysis to identify differential components relative to controls
- similar analysis aimed at smaller, more volatile target chemicals using gas chromatography-mass spectrometry (GC/MS) and gas-chromatography tandem mass spectrometry (GC-MS/MS)
- targeted analysis of mycotoxins and their metabolites and protein adducts

4.3 Clinical opinion

Clinical expert opinion was sought to describe the clinical features seen in cases and to consider the potential causes consistent with the clinical syndrome.

4.4 Epidemiological methods

4.4.1 Study population

The study population comprises of all cases included in the hepatitis of unknown origin line list to 19 July 2022. These are cases meeting the case definition criteria above.

4.4.2 Study design

Study design used:

- cross-sectional descriptive epidemiology – using the data sources described above, to explore and describe food, drink and water exposures of cases
- comparative analysis – to compare reported consumption rates to national dietary surveys and a market research panel

4.4.3 Data sources

4.4.3.1 Line List

The line list, which includes all cases notified to UKHSA to 19 July 2022, included Personal Identifiable Information, clinical information from the notification and viral exposures that could be exported from UKHSA's Second generation Surveillance System (SGSS) laboratory results. This was provided in an Excel spreadsheet updated daily.

4.4.3.2 Questionnaires

Due to the age of cases, responses to questionnaires were typically provided by parents or guardians. Questionnaires were not designed from the outset to explore environmental or food exposures and therefore data provided on exposures frequently needed to be extracted from free-text responses.

4.4.3.3 Trawling questionnaire

The trawling questionnaire outcomes were provided in an Excel spreadsheet. This data set included responses from all cases who responded to the request to complete an initial trawling questionnaire before 28 April 2022. The cases were asked about exposures, under broad categorical headings, to pharmaceuticals, pets, bodies of water, sewage, food, water and drinks. For all exposures, respondents were asked about cases exposures since 1 January 2022, giving a different exposure period for each case. All cases were also asked about their exposures in the 7 days prior to onset of illness. During the outbreak period, another outbreak of salmonella also occurred, related to a specific brand of chocolate and therefore the only specific item of food (by type, quantity and brand) prompted was this type of chocolate.

4.4.3.4 28-day questionnaire

The 28-day follow-up questionnaire outcome were also provided in an Excel spreadsheet. This questionnaire was undertaken with cases' guardians 28 days after diagnosis. It was completed by those who agreed to complete the questionnaire between 7 June and 15 July and sought to ask specifically about types of diet and milk consumption and then all food-based exposures in a single open-ended free text for the 2months before onset of illness.

4.4.3.5 Exploratory Interviews

Outcomes of the exploratory interviews were given in a separate word document. These were undertaken by scientists in the Gastrointestinal Infections and Food Safety (One Health) Division with respondents whose parents or guardians agreed to be called after June 2022. Cases were asked if they were happy to complete an exploratory interview to cover a detailed food history in the 3-month period prior to onset. The aim of the exploratory interview was to investigate the profile of cases affected, and the types of foods and diets consumed by typical cases to identify potential products of interest for further investigation.

This approach is considered suitable where a very wide range of exposures may be plausible as causal or contributory to illness and attempts to at least partially mitigate the inability of interviewees to accurately recall all specific foods eaten during the period of interest (in this case 3 months) which must be considered when assessing case exposure information derived

from case interviews. Cases are asked within the context of an open-ended discussion type format about their lifestyle, dietary habits, and a food history. Where cases were unable to recall, they answered by providing example meals they believe they ate around that time and their favourite types or brands of food often consumed. Specific food and drink types were prompted in the discussion if flagged as commonly reported from the trawling questionnaires (cucumber or bottled water and so on) or those evidenced by the literature as feasible for mycotoxin contamination (corn or wheat-based, oats, potatoes, rice or grains, nuts, fruits and dried fruits, herbs or spices, long life products or snacks).

4.4.4 Data de-duplication

There was a need for data de-duplication about cases as information was received in 3 separate Excel spreadsheets including:

- the line list
- the trawling questionnaire outcomes
- the 28-day questionnaire outcomes

Data from the 3 spreadsheets was imported into STATA as 3 separate files and merged one at a time. Information on each case was de-duplicated based on NHS number (which was sufficient alone for the line list and 28-day questionnaire), HPzone number, DOB and postcode, taken in this order. NHS numbers from the 28-day questionnaire were de-duplicated against those in the trawling questionnaire.

4.4.5 Data cleaning for descriptive analysis

To extract information about exposures, the following procedures were followed:

For the line list – a text search of variables was undertaken for any fields containing water, drink, food, exposure, or other exposure.

For the trawling questionnaire variable fields relating to food, drink and water, were reviewed one by one. The priority order for exploration of exposures was reviewed regularly and based on findings of the literature review.

- variables with a yes or no response were converted to binary 0/1 numeric fields in STATA
- variables with free field text responses were reviewed one observation at a time:
    - where there were single exposures listed (such as for type of water problem) in each observation, a string variable was used and the results tabulated
    - where multiple exposures were listed (such as for food exposures), a new variable entitled with the food exposure was generated based on presence by using STATA to look in free text for the exposure (including variations, spelling mistakes, abbreviations and capitalisation) using ‘strpos’ command. We looked in one or more fields – that is, carrot was found in the fruit and vegetable fields. If found response was converted to binary 0/1 numeric fields in STATA

    - where a food item described in free text falls into a different higher-level categories (for example, sausage rolls in desserts), the higher-level category it should be in (that is, meat), is converted to a 1 if not already
    - the overarching category which the respondent identified it in remains as a 1 as they may still have consumed other items from the overarching category
- exposures noted in the 7 days before illness onset, but not since the 1 January 2022, were assumed to also have been exposures since 1 January 2022 as all cases first presented after 1 January 2022 (as per case definition)

For the 28-day questionnaire, variable fields relating to food, drink and water were reviewed one by one. These were presented as yes or no response and not free text and were therefore easily tabulated. A text-based review (as described above was undertaken on the field entitled ‘this section has been modified’ – which is where some feedback on food exposures was contained.

4.4.6 Describing exposures.

Exposures are described by food, drink and water exposures. Counts of cases exposed and unexposed from each source are described in tabular format.

Food, drink and water exposures were described as follows:

- exposures to water including water and sewage system problems, large bodies of open water and exposure to water as a drink since 1 January 2022
- exposure to water and other drinks are described by the type, and where available brand of drink and time frame of exposure:
    - in the trawling questionnaire, this is separated into 2 time periods – since 1 January 2022 and in the 7 days before onset of illness.
    - in the 28-day questionnaire, questions are asked about exposures in the 2-months before symptom onset; this was asked 28-days after onset and therefore recall bias needs to be considered.
    - in the exploratory interviews, cases are asked within the context of an open-ended discussion type format about their lifestyle, dietary habits and a food history, about the food exposures in the 3 months preceding onset of illness that they can recall.
- exposures to food, including consideration of the types of diet cases follow and then the types and brands of food consumed; the same time periods as for drink are considered

4.4.7 Prioritising exposures.

An initial rapid review of literature by the toxicology and environmental epidemiology teams, identified potential food sources commonly associated with aflatoxins (12). Food items from this literature search were prioritised over pharmaceuticals and pets. These included:

- cassava

- maize
- noodles

A rapid review by the Global HIV Hepatitis and STI programme (13), identified the risk of hepatitis mostly from viral causes or medical conditions – however, some other exposures were noted, including:

- 2 cases in children in the United Arab Emirates (UAE) with heat shock (environmental climate exposure)
- some cases in Italy and the Netherlands with potential drug induced liver injury; the most likely causative drugs were found to be antibiotics, specifically clarithromycin, amoxicillin and co-amoxiclav

This investigation therefore focused on the most common risk factors associated with the outbreak, and did not consider pharmaceutical, pets or other exposures.

4.4.8 Determining thresholds

Exposures that exceeded a pre-determined consumption or exposure threshold were explored in more detail. The thresholds set were low in order to ensure all potentially notable exposures were captured in the initial epidemiological analysis and were set as:

- over 20% of the cases reporting exposure to the item in the trawling questionnaire or 28-day questionnaire
- where there was more than a 20% increase in consumption in the 7 days before illness onset compared to since the 1 January 2022 in the trawling questionnaire
- where there is an exposure >5% that was felt in expert consensus to be a potentially unusual finding for this age group in the trawling questionnaire
- where $\geq$5 (>50%) of the respondents to the exploratory interviews reported a specific exposure (this was set at a higher level due to the likelihood that it was more representative of actual consumption compared to the questionnaires; the questionnaire threshold was set at 20% and previous studies have shown underreporting of up to 29% (12), therefore a threshold of 20% in questionnaires and >49% for surveys is likely to be more comparable than using the same threshold for both

4.4.9 Selecting comparators

Comparators for consumption of food products are used to show if there is a higher consumption of these products compared to the general population. Ideally this would be a population sample representative of the population our cases came from, however there are a number of caveats to the sources used and these should be used as a crude indication for food items / exposures of interest only. Comparison analysis highlights the items that may need further consideration and exploration as to a potential causes or contributory exposures of hepatitis. We were able to identify 2 sources for comparison. The national dietary nutritional

survey (NDNS) and a market research panel (MRP) used as controls in a previous Salmonella outbreak.

4.4.9.1 National Diet and Nutrition Survey

National Diet and Nutrition Survey (NDNS) data estimates typical consumption of certain foods/ food groups in the general population over a 4-day time frame (14), according to defined age categories. It should be noted that underreporting is a known issue in NDNS as well as other dietary surveys (15), approximated at around 29% lower than typical consumption within the UK NDNS (14). No demographics other than age were available from the data. NDNS data was split by age group and consumption was averaged across the 1.5 to 10 year age group. The most comparable food type was selected where available or combined across several food types where not available.

4.4.9.2 Salmonella outbreak market research panel control group

Data available from market research panel (MRP) respondents was collected from 183 control questionnaires collected to inform analytical epidemiological studies carried out for recent Salmonella outbreaks in England, where the age/sex demographic for the controls data collected was approximately comparable to the hepatitis cases and specific questions covering the food items identified in the analysis of the hepatitis case exposure information was specifically covered in the control panel surveys. Respondents were selected based on reporting that the children for which the data was gathered were healthy with no history of GI illness or travel abroad in the 7 days prior to the parent or guardian answering the survey. Twenty-seven (15%) of questionnaires were completed for those aged 0 to 5 years. Respondents were asked to complete the food history survey based on exposures over a 7-day timeframe. Consumption rates of the most comparable food type was selected where available, comparators for some food items were not available from this data source

There are limitations to the representativeness of market research panels used for controls, in particular due to quality concerns, coverage bias and non-response. This may be a result of internet usage, awareness, and a tendency to rush through the questionnaire without full completion of all the required data fields. Additionally, there are caveats to be considered around the structure and stratification of respondent selection where MRP data was collected for other reasons and is now being applied as a comparator to the hepatitis case data.

4.4.10 Comparative analysis

Where the denominator and numerators for the data sources were known (both for cases and comparative data sources), 95% confidence intervals on the proportion of cases or controls who consumed the food item were calculated. Where case consumption exceeded the consumption rates identified in the comparator data sets the 2-sample z- test of proportions was undertaken to compare the proportions and a p value calculated to determine if any exposures were statistically significant at the $p<0.05$ value.

5. Results

5.1 Literature review results

5.1.1 Toxicological literature review results

The majority of data available on the hepatotoxicity of mycotoxins in humans is on aflatoxins. The clinical presentation of acute aflatoxicosis can be varied making it difficult to diagnose. Symptoms include vomiting, abdominal pain, anorexia, malaise, low-grade fever, pulmonary oedema and acute liver failure. There is some experimental animal data to suggest that some other mycotoxins may be hepatotoxic including cyclopiazonic acid, fumonisins, patulin, ochratoxin, sterigmatocystin and zearalenone. Table 1 details further information on these mycotoxins identified as causing liver toxicity, and the names of mycotoxins included in the search strategy (Appendix 1).

Table 1. Toxicological literature search results, showing Mycotoxin and further information

Cyclopiazonic acid

After ingesting cyclopiazonic acid contaminated feeds, test animals display gastrointestinal and neurological effects. Organs affected include the liver, kidney, heart, and digestive tract, which show degenerative changes and necrosis. There is little evidence available for toxicity in humans due to consumption of food contaminated with cyclopiazonic acid (16).

Fumonisins

Toxicity assessments are mainly based on fumonisin B1, fumonisins B2-4 are considered as having a similar toxicological profile and potency as fumonisin B1. Fumonisin B1 is not considered to be acutely toxic in humans (17, 18).

Patulin

Patulin causes liver inflammation (inducing a rise in alkaline phosphate (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST) and malondialdehyde (MDA)) in rodents (16, 20). Liver enlargement, inflammation and cellular necrosis were observed in male albino mice following oral administration of patulin 0.1 mg/kg body weight for 3 weeks (20).

Ochratoxin

Ochratoxin A has been reported to be hepatotoxic in laboratory animals (10). Fatty infiltration, hyaline degeneration and focal necrosis has been observed in rats following a single oral dose of ochratoxin A (21).

Sterigmatocystin

The liver appears to be a target organ for acute exposure in experimental animals (22). Hepatocarcinogenicity observed in experimental animals administered sterigmatocystin via oral

route (18). High levels of sterigmatocystin have been observed in the urine of humans with liver cirrhosis (22).

Zearalenone

Studies devoted to subacute and subchronic toxicity of zearalenone indicate that haematological changes, hepatic disturbances and oestrogenic effects are observed in rodents receiving high oral doses of zearalenone. There is some limited evidence of carcinogenicity of the liver (17).

There is little information on single acute aflatoxin exposure; however, 8 cases or outbreaks of reported aflatoxicosis were identified in the literature. These are described in Table 2 below.

Table 2. Overview of reported outbreaks or cases of aflatoxicosis (23 to 26)

Country and year	Number of subjects	Signs and symptoms	Source	Toxin and food sample concentration	Duration of exposure	Liver histopathology
Uganda 1970	1	Abdominal pain, oedema of legs, palpable liver, abnormal ECG	Cassava	Aflatoxin 1.7ppm	5 to 30 days	Centrilobular necrosis, polymorphonuclear infiltration and fibrin in sinusoids, fatty changes in midzonal region.
India 1974	397	Brief febrile episode, vomiting, anorexia, jaundice, ascites, oedema of the legs, massive gastrointestinal bleeding	Maize	Aflatoxin B_1 6.25 to 15.6 ppm detected in 5 out of 5 samples	Several weeks	Bile duct proliferation with periductal fibrosis, multinucleated giant cells, foamy cytoplasm, bile stasis in bile ducts, dilated biliary canaliculi.
India 1974	994	Fever, vomiting, oedema of feet, jaundice, hepatomegaly, ascites, splenomegaly	Maize	Aflatoxin B_1 0.01 to 1.1 ppm Detected in 13 out of 14 samples	Unknown	Cholangiolar proliferation, perivenous collagenosis, luminal obliteration, extensive fibrosis, giant cell transformation of hepatocytes, moderate to severe cholestasis and proliferation of bile ducts
Kenya 1981	20	Brief febrile episode, vomiting, abdominal discomfort, anorexia, jaundice, oedema of legs, ascites, tachycardia, tenderness of liver, melaena, GI bleeding	Maize	Aflatoxin B_1 3.2 to 12 ppm Detected in 2 out of2 samples Aflatoxin B_2 1.6 to 2.7 ppm Detected in 2 out of 2 samples	Several weeks	Marked centrilobular necrosis, slight fatty infiltration and no proliferation of bile ducts
Kenya 2004	317 Age data available for 308 cases: 68: < 5yrs 90: 5-14 yrs 150: ≥ 15 yrs	Jaundice, pulmonary oedema	Maize	Aflatoxin B_1 Detected in15/31 samples at a concentration > 20 ppb (Range 20 – 8000 ppb)	Unknown	Unknown
Kenya 2004 (possibly a case study of the outbreak in Kenya	1 (17 year old) 7 other children in the school had milder symptoms	Vomiting, lethargy, body aches, severe abdominal pain, bruising and bloody diarrhoea	Maize	-	At least 6 days	Enlarged liver and dilated bile ducts
USA 1966	1 2 episodes of intentional ingestion	First ingestion: non-pruritic macular rash, nausea, headache Second ingestion: nausea	Purified aflatoxin B1	Ingested 5.5 mg dose over 2 days 6 months later ingested 35 mg over 2 weeks		Normal

Country and year	Number of subjects	Signs and symptoms	Source	Toxin and food sample concentration	Duration of exposure	Liver histopathology
Malaysia 1988	17 (16 children (mean age 7) and 1 adult)	Vomiting and seizures, pyrexia, diarrhoea, abdominal pain, anorexia, mild jaundice	Rice and corn flour noodles – also contaminated with boric acid	-	Mean incubation period between ingestion and onset of symptoms was 8.5 hours	Centrilobular necrosis with proliferative ductal metaplasia of the hepatocytes. Renal necrosis was also present, likely to be due to the boric acid

5.1.2 Epidemiological literature review results

The initial search, including all the terms identified in box 2, identified 3,316 papers. We refined the search to exclude further terms such as fungus, mushroom, and transplantation. This reduced the number of papers down to 1,437 papers. We screened papers using the title and abstracts and overall, across all 4 searches, 54 of the papers were identified as potentially relevant. Search 2 and 4 (as detailed in box 1) produced the greatest number of results (Table 3). There were a few papers on exposure to mycotoxins and inclusion body hepatitis in poultry (a disease of young broilers caused by fowl adenovirus), which can be caused by avian adenoviruses. Most of the papers on aflatoxins and environmental exposure were on toxicity in humans. Search 4 identified some papers which discuss the recent outbreak of hepatitis in children, including possible causes such as adenovirus and SARS-CoV-2.

Most of these papers discussing the recent outbreak were in the USA, with a few in Europe and one in Scotland. As no exclusions based on date of publication were put on the search, several relevant papers published were from the 1970s.

Table 3. Literature search results

Source	Search 1: Joint effect of adenovirus and mycotoxins	Search 2: Effect of mycotoxins alone on liver function (hepatitis)	Search 3: Joint effect of mycotoxins with other contaminants or viruses	Search 4: Joint effect of adenovirus and any other contaminant or virus
Embase	1	561	242	284
Medline	4	66	23	49
Scopus	0	4	4	199
Potentially relevant papers (title and abstract screen)	3	24	2	25

Additional papers identified by experts in their fields included those showing plausibility of exposure to hepatoxic pesticides through food consumption. Further work is needed to summarise the findings of the literature review and identify other aspects to explore in the future.

5.2 Analytical chemistry results

ICPMS analysis targeted 63 elements and found none to be elevated to a level suggestive of concern. LC-HRMS analysis showed clear separation of cases and controls using 3 column chemistries and both positive and negative ionisation. Interrogation of the data found that pathological (bilirubin, bile salts, bile acids) and therapeutic (ursodeoxycholic acid, fluconazole, trimethoprim, sulfamethazole, vitamins K and D) chemicals contributed to this separation with no clear identification of potential environmental chemicals linked to hepatotoxicity. Targeted investigations of paracetamol and its metabolites found levels in the serum of some cases and controls with levels consistent with therapeutic use of paracetamol. These results were confirmed in urine by GC-MS/MS.

5.3 Clinical opinion results

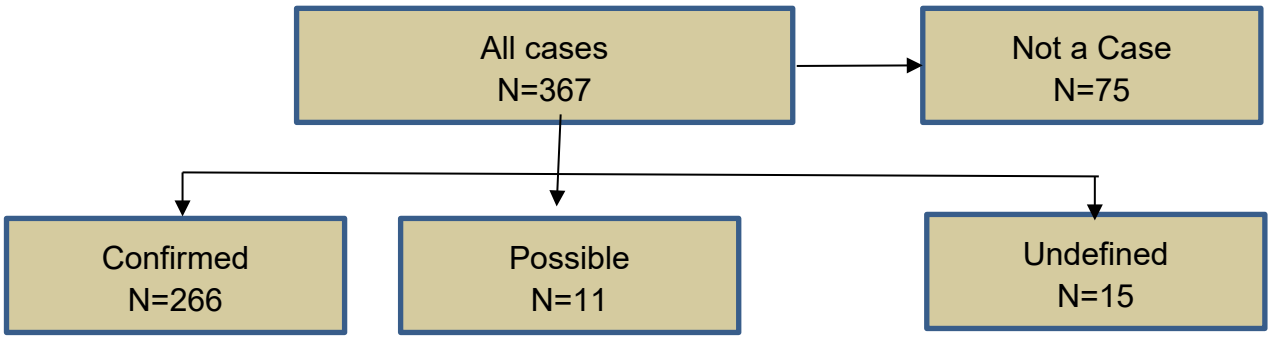
The most common presentation when cases presented to healthcare included jaundice followed by vomiting, pale stools. Cases presented with diarrhoea, nausea and abdominal pain. Additionally, lethargy, fever and less frequently, respiratory symptoms were reported. These symptoms are consistent with adenovirus (and other) infections in children, although there is a lack of an apparent direct toxic effect of the virus on the liver and therefore this may be part of a multi-step process. These symptoms are also seen with mycotoxin exposure (especially aflatoxicosis).

5.4 Epidemiological results

5.4.1 De-duplication

The line list contained 367 results, 266 confirmed cases, 11 possible cases and 75 non- cases. There were a further 15 cases with their case definition status undefined. There was no overlap of case ID's or NHS numbers (Figure 5). These numbers had changed slightly to 270 confirmed cases by the time of the Sitrep released on 19 July 2022 (3).

Figure 5. Breakdown of individuals included in line list to 19 July 2022



A total of 99 observations from the confirmed cases were included in the trawling questionnaire data. The data set included all results uploaded into the spreadsheet up to the 18 May 2022. Questionnaire completion dates ranged from 12 April to 24 April 2022. After de-duplication 77 observations remained. Of these, 72 had a unique NHS number identifier. The remaining 5 observations had unique identifier fields missing and did not have any points of commonality with each other or the remaining 72 observations and therefore were deemed to be separate cases.

41 unique observations were identified in the 28-day questionnaire. On comparison with the trawling questionnaire, there was overlap of 3 cases. In 2 of these observations, data on exposures was missing and in the third matched exposures were already identified in the trawling questionnaire. These were therefore removed from the 28-day questionnaire results to prevent duplication in presentation of data on the same case.

Exploratory interviews were undertaken with 9 cases: it is likely that these were 9 cases from the 28-day questionnaire, but these data sources came separately and have not been de-duplicated against each other. Data on patient identifiable information from the exploratory interviews was not available at the time of this report. Therefore, total counts should not be added together.

5.4.2 Demographics of cases in trawling questionnaire and 28-day questionnaire data lists

Gender

Of the 77 parents and guardians who responded to the trawling questionnaire, data on gender of cases was given for 73 (95%) cases. Gender proportions for each data source is shown in Tables 4a and 4b. Demographic data for those included in the exploratory interviews is not currently available.

Age

All respondents (confirmed and possible cases) to the trawling questionnaire reported date of birth and age was calculated from this. Ages ranged from 0 to 10, with a median age of 3 (IQR 2 to 4). In the 28-day questionnaire age ranged from 0 to 15, with a median age of 5 (IQR 3 to 5). Demographic data for those included in the exploratory interviews was not available. Age and gender of cases is shown for the trawling questionnaire and 28-day questionnaire in Tables 4a and 4b below.

Table 4a. Age and gender of cases from trawling questionnaire

Age	Female	Male	Total
0 to 5	37	29	66
6 to 10	<5	>5	7

Table 4b. Age and gender of cases from 28-day questionnaire

Age	Female	Male	Total
0 to 5	11	17	28
6 to 10	<5	>5	7
11 to 15	<5	<5	<5

5.4.3 Food and water exposures results

In this section the question numbers from the trawling questionnaire to which data is related are reported in brackets next to the point they are mentioned. These are the question numbers from version 1 of the Non-Hep A-E acute hepatitis Trawling Questionnaire (4). Questionnaire numbers are not included next to data from the 28-day questionnaire as these were asked as

yes or no responses, not as part of a free-text response. No exposure data relating to food, drink or water exposure was identified in the line list.

5.4.4 Water-based exposures

5.4.4.1 Problems with the water or sewage system

No timeframe for exposure to a water or sewage problem was requested in the trawling questionnaire (Q12c). Less than 5 cases who responded to this question reported problems with their water or sewage supply This included problems with the water system, pressure and sewage system (Q12d). Questions on exposure to water sources were asked in the 28-day questionnaire or exploratory interviews.

5.4.4.2 Large bodies of water

Of the 67 responses to this question, 9 (13%) reported exposure to a large body of water since 1 January 2022 (some to more than one type of large body of water (Q12f)). These 9 respondents reported exposures to streams, lakes, beach, puddles and private (at-home) swimming pools and hot tubs. There was no geographical overlap of sites of exposure to water bodies Questions on exposure to large bodies of water were not asked in the 28-day questionnaire or exploratory interviews.

5.4.5 Drinks including water consumption

5.4.5.1 Bottled water

Twenty-nine (44%) of 66 respondents who answered this question in the trawling questionnaire (Q12g), reported drinking bottled water (Table 7a) since 1 January 2022. Cases drank both plain and flavoured water. Less than 5 respondents drunk more than one type of water. Bottled water was consumed by more than half of the respondents interviewed for the exploratory questions. When broken down by brand, no more than 5 people identified the same brand of water. Bottled water was not asked about in the 28-day questionnaire. This compares to a national average of 16.1% in this age group (14).

Table 5. Exposure to bottled water

Exposure	Trawling Q: Since 1 January 2022: Yes (%)	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)	Trawling Q: 7 days prior to illness No (%)	Exploratory interviews: 3 months prior to illness[1]
Bottled water (any)	29 (44)*	37 (56)*	12 (31)*	27 (69)*	≥5

[1] Only a number is provided where the value is <5.

* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

Twelve respondents of the 39 who responded, reported consuming bottled water in the 7 days prior to symptom onset. Of the 10 respondents who reported on the frequency of bottled water consumption, bottled water was consumed a median of 4.5 days a week, with a range of 1 to 7

days a week. Data on frequency of consumption is not reported in a separate table due to small numbers. Questions on the frequency of consumption of bottled water were not asked in the 28-day questionnaire or exploratory interviews.

5.4.5.2 Squash and fruit juices

Drinks other than water were not specifically asked about in the trawling questionnaire; however 11 (14%) and 13 (17%) of all respondents to the trawling questionnaire (Q13d, 13l and 13l) reported drinking juice and squash in other areas of the questionnaire respectively since 1 January 2022. Consumption of drinks other than water was reported by less respondents in the 7 days prior to illness onset. Five (6%) reported drinking smoothies since January, and less than 5 continued drinking smoothies in the 7 days prior to illness onset (Table 6) Drinks other than water were explored in more depth in the exploratory interviews, with examples of fizzy drinks and milk or milk replacements given (milk consumption is discussed in the next section). Questions on consumption of squash and fruit juices were not asked in the 28-day questionnaire.

Table 6. Exposure to squash and fruit juice

Exposure	Trawling Q: Yes since 1 January 2022[1]	Trawling Q: Yes in 7 days prior to illness[1]	Exploratory interviews: 3 months prior to illness[1]
Fruit juice (any)	11	6	<5
Apple juice (any)	<5	<5	<5
Orange juice (any)	<5	<5	<5
Squash (any)	13*	8*	>5*
Smoothies (any)	5	<5	<5
Fruit pouches (any)	<5	<5	<5
Fizzy drinks (any)	<5	<5	<5

[1] Only a number is provided where the value is >5 or no denominator was available (because responses were based on comments not a specific question).

* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

5.4.5.3 Milk and dairy alternatives

No data was collected on other drinks in the trawling questionnaire including dairy and dairy alternative based drinks. Some information was identified in free text fields for other foods consumed, especially in the dietary requirements section. Comments included:

- dairy substitution with oat milk
- dairy substitution with almond milk
- dairy substitution with a prescription formula from the GP (prescription not named)

This accounts for less than 5 respondents who reported a dairy or lactose free diet (see diet section).

Consumption of any dairy milk, both full fat and semi-skimmed was reported by 26 (90%) of respondents in the 28-day questionnaire and by 7 (78%) of the respondents in the exploratory interviews (Table 7).

The section on dietary needs and patterns was only included once in the questionnaire at the time questions were asked about food consumption since 1 January 2022. Table 7 below therefore does not have a column on consumption in the 7 days prior to onset of illness.

Table 7. Exposure to dairy and dairy alternatives

Exposure	28-day questionnaire – 2 months prior to illness 5: Yes (%)[1]	28-day questionnaire – 2 months prior to illness 5: No (%)	Exploratory Interviews – 3 months prior to illness[1]
Full fat dairy milk	14 (44)*	18 (56)*	<5
Semi-skimmed dairy milk	6 (18)	26 (82)	<5
Formula (all brands)	<5		-
Breastfed only	<5		<5
Dairy substitutes	<5		-

[1] A number is only provided where the value is >5, or no denominator was available (because responses were based on comments not a specific question).
* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

5.4.6 Special dietary requirements exposures

Fourteen (21%) respondents of the 65 who answered this question (Q13a) in the trawling questionnaire reported a specific diet or dietary need. This was not a specific question in the questionnaire and therefore data is assumed missing/unknown for all other respondents. It was also only asked once in the since 1 January 2022 set of questions and is therefore no column for the 7 days prior to onset of illness is included in Table 8 below. Specific diets reported included Halal, vegetarian and vegan, whilst some reported following a specific allergen free diet, or solely breastfeeding (Table 8).

In the 28-day questionnaire this question was expanded to ask about any allergies or intolerances. All respondents answered this question (n=38) and of these, 12 (30%) identified an allergy, 5 to a medication, less than 5 to pollen (hay fever), nuts, wheat, lactose or more generally to dairy. Less than 5 reported lactose- or dairy-free diets.

Table 8. Reported specific dietary requirements

Exposure	Trawling Q: since 1 January 2022: Yes (%)	Trawling Q: since 1 January 2022: No (%)	28-day Q: question not asked*	Exploratory interviews: 3 months prior to illness*
Specific diet	14 (21)*	51 (78)*	-	<5
Dairy free	5 (9)*	60 (91)*	<5	<5

[1] A number is only provided where the value is >5, or no denominator was available (because responses were based on comments not a specific response to the question on allergens).
* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

Dairy-free and lactose-free diets are similar, and a vegan diet would also be dairy free, and many of the alternatives offered are the same for these diets. Considered together as a proportion of those who responded to this question (n=65), consumption of a dairy free diet is the preference of 8 (12%) respondents. Less than half of the respondents to the exploratory interviews reported either a dairy free diet or an intolerance to full fat cream. Very few of the 38 28-day questionnaire respondents reported any special diets, but of these dairy free was the most common, but still less than 5 respondents. Exposure to this type of diet from these 2 sources was much lower than seen in the trawling questionnaire.

5.4.7 Food exposures

5.4.7.1 Vegetable consumption

Raw and uncooked vegetables were asked about in the trawling questionnaire (Q13g), but many reported other vegetable consumption in other sections of the questionnaire – such as broccoli, carrots, sweetcorn, peas and cucumber in the fruit and ‘other’ sections. It is not clear why, but it may be that these were in smoothies – note the specific sub-category of smoothie in Q13d on fruit. A specific question in the trawling questionnaire was designed to ask about sundried tomato consumption (Q13f).

Table 9. Exposure to vegetables and salad items

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory Interviews:3 months prior to illness[1]
Sundried tomatoes	<5		1 (3)	37 (97)	<5
All vegetables	45 (69)*	20 (31)*	28 (68)*	13 (32)*	≥5
Cucumber	29 (52)*	27 (48)*	14 (45)*	17 (55)*	≥5
Tomatoes	17 (30)*	40 (70)*	9 (28)*	23 (72)*	<5
Carrot	14 (25)*	42 (75)*	6 (19)	25 (81)	≥5

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory Interviews:3 months prior to illness[1]
Potatoes	<5		<5		**≥5**
Chips[2]	<5		<5		**≥5**
Peas	<5		0		**≥5**
Broccoli	<5		0		**≥5**
Sweetcorn	<5		<5		**≥5**
Avocados	-	-	-	-	<5
Peppers	8 (14)		<5		<5
Lettuce	<5		<5		-
Cauliflower	<5		<5		<5
Squash	<5		<5		-
Pumpkin	<5		<5		-
Green beans	<5		<5		-
Olives	<5		<5		-
Spring onions	<5		<5		<5
Celery	<5		0		-
Spinach	<5		<5		-
Sweet Potato	-	-	-	-	<5
Seaweed[2]	<5		<5		-
Humus[2]	<5		-	-	-

[1] A number is only provided where the value is >5, or no denominator was available (because responses were based on comments not a specific question).

[2] Data on seaweed, chips and humus (main constituent: chickpeas) was captured from comments in a different question.

* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

Using the methodology outlined above, the most commonly reported vegetable and salad items consumed since 1 January 2022 identified on the trawling questionnaire were cucumber (52%), tomatoes (30%) and carrots (25%). Cucumber and tomatoes consumption continued to be reported above 20% in the 7 days before illness onset (45% and 25% respectively). Consumption of vegetables, including cooked vegetables was explored in more depth in the exploratory interviews. In the exploratory interviews tomatoes were not as commonly reported, whilst potatoes, carrots, cucumber, broccoli, sweetcorn and peas were all reported to be consumed by 5 (56%) or more of the exploratory questionnaire respondents. Consumption of

vegetables was poorly explored in the 28-day questionnaire, with no specific questions about vegetable or salad consumption asked.

5.4.7.2 Herb consumption

Of the 63 respondents to the trawling question (Q13h) covering exposures since 1 January 2022 and 41 in the 7 days before illness onset, 13 (21%) and 7 (17%) respectively reported herb consumption. Most did not know the specific details of the herbs consumed (Table 10). Herb consumption was not explored in the 28-day questionnaire and therefore not included in Table 10 below. Paprika was the most commonly reported herb amongst exploratory interview respondents. Table 10 also reports world foods which may have included herbs or sauces with herbs. Data on these food types was captured in a later question.

Table 10. Exposure to herbs and spices and world foods or their sauces which may contain herbs or spices

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory interviews: 3 months prior to illness[1]
Any herbs	13 (21)*	50 (79)*	7 (17)*	34 (83)*	≥5*
Unknown herbs	5 (7)	58 (93)	<5		<5
Paprika	-	-	-	-	<5
Curry[2]	<5		-	-	<5
Chinese[2]	9 (14)	54 (86)	-	-	<5
Indian[2]	<5		-	-	<5
Thai[2]	<5		<5		-
Turkish[2]	<5		-	-	-

[1] A number is only provided where the value is >5.

[2] Consumption of these items was captured in a different question on specialty foods.

* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

5.4.7.3 Fish, shellfish or mollusc consumption

A total of 66 (86%) respondents answered this question on the trawling questionnaire (Q13g) when considering any fish consumption since 1 January 2022, with 41 (53%) answering this question about the 7 days before illness onset. Thirty-eight respondents (58%) consumed fish of any type since 1 January 2022 and 21 (51%) in the 7 days before symptom onset. Consumption of fish fingers was higher than 20% both since 1 January 2022 and in the 7 days before illness onset and this was the fish consumption reported in the exploratory interviews by more than 5 respondents (Table 11). All of those in the exploratory interviews who ate fish, ate fish fingers. Fish consumption was not a question asked in the 28-day questionnaire and is therefore not included in Table 11 below.

Table 11. Exposure to fish based products

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory interviews: 3 months prior to illness[1]
To any fish	38 (58)*	28 (42)*	21 (51)*	20 (49)*	≥5*
Fish fingers	19 (29)*	46 (71)*	7 (20)*	29 (80)*	≥5*
Salmon	11 (17)	53 (82)	5 (14)	31 (86)	-
Cod	11 (17)	54 (84)	5 (14)	31 (86)	<5
Tuna	<5		<5		<5
Haddock	<5		0		-
Fish pie	<5		<5		-
Fishcake	<5		0		<5
Mackerel	<5		<5		-
Any shellfish	<5		<5		-
Scampi	<5		0		-
Prawn	<5		<5		-
Crab sticks	<5		<5		-

[1] A number is only provided where the value is >5.
* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

5.4.7.4 Meat consumption

A total of 66 (86%) respondents answered this question in either the meat section of the trawling questionnaire (Q13h) or with sausage rolls identified as a pastry in the desert section. Those who reported in the comments that no meat was consumed but only gelatine in jelly sweets were reclassified as negative. Of those who responded, 59 (91%) reported eating meat since 1 January 2022, with consumption of any chicken, any beef or any pork product over 20%. Likewise in the 7 days before illness onset, 36 (88%) reported eating meat. Of the specific products identified with higher consumption, sausages and ham were consumed by more than 20% of respondents both since 1 January 2022 and in the 7 days before illness onset.

In the trawling questionnaire, sausage rolls were consumed by 8 (13%) respondents since 1 January 2022, but consumption increased based on proportion to 7 (21%) of overall responses in the 7 days before illness onset. Likewise, consumption of gammon increased from 11% to 18% in the last 7 days. Meat consumption was not specifically asked about in the 28-day questionnaire, but some respondents gave free-text responses to questions which included comments on meat exposures.

All of the exploratory interview respondents reported consumption of meat and chicken products. Within each type of meat product, particular items were consumed by more than 5 respondents, these included chicken nuggets, beef mince, pork sausages and ham.

Table 12. Exposure to meat products

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory Interviews: 3 months prior to illness[1]
To any meat	59 (91)*	6 (9)*	36 (88)*	5 (12)*	≥5*
To any chicken	45 (74)*	16 (26)*	22 (65)*	12 (35)*	≥5*
Chicken nuggets	8 (13)	53 (87)	<5		≥5*
To any beef	26 (43)*	35 (57)*	13 (38)*	22 (62)*	≥5*
Mince	11 (18)	50 (82)	6 (17)	28 (83)	≥5
Burger	<5		<5		-
Meatballs	<5		<5		<5
Sausages	<5		0		-
To any pork	39 (64)*	22 (36)*	22 (65)*	12 (35)*	≥5*
Bacon	<5		<5		<5
Pork pie	<5		0		-
Sausage	24 (39)*	37 (61)*	14 (41)*	20 (59)*	≥5*
Sausage roll[2]	8 (13)	53 (87)	7 (21)*	27 (81)*	<5
Ham	15 (25)*	46 (75)*	8 (24)*	26 (76)*	≥5*
Gammon	7 (11)	54 (89)	6 (18)*	28 (82)*	-
To any lamb	8 (13)	52 (87)	<5		<5
To any turkey	<5		<5		<5

[1] A number is only provided where the value is >5.
[2] Fewer than 5 individuals identified sausage rolls as a desert, not meat. Totals include those who identified sausage rolls in the dessert section.
* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

5.4.7.5 Other and specialty foods

A total of 64 (83%) respondents answered questions (Q13j) about specialty food in the trawling questionnaire. No types of food stood out, and in the exploratory interviews some respondents identified curry as a speciality food. Specialty foods were not identified in the free-text responses of the 28-day questionnaire.

Table 13 below shows exposures to rice, grains, cereals and pasta. Some respondents to the trawling questionnaire identified rice as a specialty food, but rice and pasta are also picked up in different places in the trawling questionnaire, and under the category of other in the 28-day questionnaire. They are explored as specific food exposures in the exploratory interviews. In the interviews these were reported to be consumed by the majority of respondents.

More than half exploratory interview respondents reported consuming bread containing wheat cheese (cheddar), chips and tortilla wraps. Tortilla wraps had not been specifically asked about or reported in free text comments in any other data sources. Most respondents reported consumption of cereal, but this was a variety of types with different bases – that is, wheat, corn, or oats. Only consumption of pasta, bread and rice crispies was identified in the 28-day questionnaire, and with less than 5 respondents consuming it is not included in the tables below.

Other foods not elsewhere captured in the tables above from either the trawling questionnaire, 28-day questionnaire or exploratory interviews are detailed in Table 14 (Q13l). Proportions can only be calculated amongst those involved in the exploratory interviews as these were not food exposures asked about or explored in the trawling or 28-day questionnaire.

Table 13. Rice, grains, cereals and pasta exposures

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory Interviews: 3 months prior to illness[1]
Rice[1]	<5		-	-	≥5*
Egg fried rice	<5		<5		-
Pasta[1]	6	-	<5		≥5*
Noodles	<5		0		-
Bread[1]	<5		-	-	≥5*
Brioche**	<5		<5		-
Any pastry**	13 (23)*	43 (77)*	6 (20)*	24 (80)*	≥5
Batter (crepe, pancake, waffle)[2]	<5		<5		<5
Croissant[2]	<5		0		<5
Pizza	<5		-	-	<5
Tortilla wraps	<5		-	-	≥5*
Popcorn	<5		-	-	-
Porridge[1]	<5		-	-	≥5*
Cereals (any)[1]	<5		-	-	≥5*

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory Interviews: 3 months prior to illness[1]
Shreddies[1]	<5		-	-	<5
Cornflakes[1]	<5		-	-	<5
Rice crispies	-	-	-	-	<5
Cheerios or hoops	-	-	-	-	<5
Weetabix	-	-	-	-	<5

[1] A number is only provided where the value is >5.

[2] Pastry items and brioche were noted under desserts and pastries in the trawling questionnaire and have been added to this section.

* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

Table 14. Other food items not identified elsewhere

Exposure	Trawling Q: since 1 January 2022[1]	Trawling Q: since 1 January 2022[1]	Trawling Q: 7 days prior to illness[1]	Trawling Q: 7 days prior to illness[1]	Exploratory Interviews: 3 months prior to illness[1]
Cheese	<5		-	-	≥5*
Eggs	<5		<5		<5
Peanuts	-	-	-	-	<5
Mushrooms	-	-	-	-	<5

[1] A number is only provided where the value is >5.

* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

5.4.7.6 Fruit consumption

Fifty-eight (75%) cases of the total 77 who responded to the trawling questionnaire, reported consumption of fruit in the fruit section (Q13e) alone (separate to just fruit juice or fruit smoothies which are detailed in the drinks section above) since 1 January 2022. However, many also identified fruits consumed in the desserts, sweets and treats and ‘other’ section. Therefore, in total 66 respondents to the trawling questionnaire responded to questions about fruit consumption. 63 (95%) reported consumption of fruit since 1 January 2022 and 44 (93%) were still consuming fruit in the 7 days prior to onset of illness. Many respondents consumed more than one type of fruit, the number of cases consuming each fruit is recorded in Table 15 below. Fruit consumption is not included in the 28-day questionnaire. It was explored in more detail in the exploratory interviews with many of the same fruits identified as high consumption items in the trawling questionnaire also being reported to be consumed by more than 5 of the 9 respondents to the exploratory interview.

Table 15. Exposure to fruit

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory interviews: 3 months prior to illness[1]
Any fruit	63 (95)*	-	44 (93)*	-	≥5*
All fresh	55 (85)*	10 (15)*	33 (87)*	5 (13)*	≥5*
Grape	21 (32)*	43 (67)*	15 (39)*	23 (61)*	<5
Banana	32 (49)*	33 (51)*	18 (47)*	20 (53)*	≥5*
Orange	19 (30)*	44 (70)*	12 (32)*	26 (68)*	<5
Apple	27 (42)*	37 (78)*	16 (42)*	22 (58)*	≥5*
Pear	9 (14)	55 (86)	6 (16)	32 (84)	<5
Mango	<5		<5		<5
Watermelon	<5		<5		<5
Melon	<5		<5		<5
Pineapple	<5		<5		<5
Plum	<5		0		0
Kiwi	<5		<5		0
Dried fruit (any)	9 (14)	54 (86)	6 (16)	32 (84)	≥5*
Berries (any)	30 (48)*	33 (52)*	18 (47)*	20 (53)*	≥5*
Strawberry	23 (37)*	40 (63)*	13 (34)*	25 (66)*	≥5*
Raspberry	5 (8)	58 (92)	5 (13)	33 (87)	<5
Blackberry	<5		<5		0
Blueberry	7 (11)	56 (89)	4 (11)	34 (89)	<5
Cranberry	<5		<5		-

[1] A number is only provided where the value is >5.

* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

Fruits with a consumption rate, from the trawling questionnaire, over 20% include grape, banana, apple, berries (any), strawberry, and orange. These 5 fruits remained the most commonly reported fruits (more than 20%) in the 7 days before onset of illness. However, only bananas, apples and strawberries were reported to be consumed by 5 or more of the exploratory interview respondents. Bananas were also reported to be consumed by all respondents to the exploratory interviews.

5.4.7.7 Dessert consumption

A total of 65 (84%) respondents to the trawling questionnaire answered this question, but not all gave a text description and of those who did, not all responses related to the specific dessert items requested (cake, pastries and puddings). The number of respondents may therefore be

underreported especially where they were not prompted to think about certain food items. Only consumption of yoghurts (and no other dessert items) was asked about in the 28-day questionnaire. Questions about dessert consumption were asked to the exploratory interview respondents, but with different prompts. Cakes, biscuits and pastries were included in the dessert prompts in the trawling questionnaire but are presented under bread products.

Cake was consumed by 48% of trawling questionnaire respondents and most of those who responded to the exploratory interviews. Consumption of different types of cake gave several different brands and example types given. Fairy cakes and Brand E cakes were the most commonly reported types and brands of cake, consumed by less than 5 trawling questionnaire respondents when expansion was given in the free text on ‘types of cake’.

Biscuits were consumed by 11 (20%) trawling questionnaire respondents since 1 January 2022. These were asked about in only the dessert section but are reported in both the dessert and sweets and treats sections. However, the variety of biscuits consumed was wide. When the types and brands of biscuits consumed by exploratory interview respondents was also considered the variety eaten was even wider.

Some respondents from the exploratory interviews reported consumption of ice cream and yoghurt. This was not seen in data captured in the trawling questionnaires with less than 5 reporting these in the dessert section. This may reflect the way in which the dessert question was asked, and with no specific prompts about ice cream or yoghurts and may demonstrate significant underreporting. Yoghurts were the only dessert item captured in the 28-day questionnaire however, this also came from free text responses and not a binary specific question.

5.4.7.8 Sweets and treats

A total of 65 respondents to the trawling questionnaire answered this question. Of these 61 (93%) ate sweets. Of these, 59 (91%) ate chocolate and 19 (53%) ate Brand B since 1 January 2022. There are however lots of different types of sweets manufactured by Brand B and it is not clear what types of sweets respondents are referring to. Of the chocolates consumed, 23 (40%) ate Brand C – however, the trawling questionnaire was undertaken around the Easter period and during a time when Brand C products were in the media because of a recall – and so there may be some recall bias as this is not seen in the later exploratory interviews. On further investigation, it was also noted that interviewers were asked by the IMT to specifically prompt about Brand C products whilst completing the trawling questionnaire – there is therefore also an issue of reporting bias. Brand D was the secondly most consumed chocolate by 17 (30%) trawling questionnaire respondents. This had fallen in the 7- days before symptom onset.

Sweet and chocolate consumption was not explored in the 28-day questionnaire.

The period since 1 January 2022 for many cases included Easter – Easter may not have been included in the 7 days before symptom onset. It should have been captured in the 2- and 3-month periods pre-illness for many of the 28-day questionnaire and exploratory interview

respondents respectively. This should be considered when interpreting the findings of chocolate consumption.

Table 16. Exposure to puddings and snacks (including cakes and non-chocolate biscuits)

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory interviews: 3 months prior to illness[1]
Any cake	27 (48)*	29 (52)*	15 (50)*	15 (50)*	>5*
Fairy cake	<5		<5		<5
Chocolate	6 (11)	49 (89)	<5		<5
Choc brownie	<5		<5		<5
Muffins	<5		<5		<5
Doughnut	<5		<5		<5
Pudding: custard tart	<5		0		-
Pudding: ice cream	<5		0		<5
Pudding: yoghurt	<5		<5		≥5*
Pudding: jelly	<5		0		<5
Non chocolate biscuits: biscotti	<5		<5		<5
Non chocolate biscuits: wafers	-	-	-	-	<5

[1] Only a number is provided where the value is <5.

* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

Table 17. Sweets and chocolate biscuits

Exposure	Trawling Q: Since 1 January 2022 Yes (%)[1]	Trawling Q: Since 1 January 2022 No (%)	Trawling Q: 7 days prior to illness Yes (%)[1]	Trawling Q: 7 days prior to illness No (%)	Exploratory interviews: 3 months prior to illness[1]
To any sweets	61 (94)*	4 (6)*	34 (83)*	7 (17)*	<5
Brand B (any product)	19 (53)*	17 (47)*	6 (38)*	10 (62)*	<5
To any chocolate (not biscuits)	59 (91)*	6 (9)*	31 (76)*	10 (24)*	≥5*
Brand D product A	17 (30)*	40 (70)*	<5		<5
Brand C	23 (40)*	34 (60)*	10 (34)*	19 (66)*	<5
Brand D product B	11 (19)	46 (81)	<5		<5
Chocolate biscuits[2] Brand E	<5		<5		<5
Chocolate biscuits[2] choc cookies	<5		-	-	<5
Chocolate biscuits[2] Brand F	<5		<5		-

[1] Biscuits were asked about in the dessert and pastries section. Respondents were asked about biscuit consumption and then separately asked about chocolate consumption. There could therefore be confusion as to how these products were categorised by respondents and then recorded.
[2] A number is only provided where the value is >5.
* An asterisk indicates exposures or consumption greater than the predetermined thresholds.

5.4.8 Exploratory questionnaire summary

Cases were contacted to complete an exploratory food history interview covering the approximate 3-month period prior to onset of acute hepatitis. Nine respondents were available by phone and completed an interview. To note: exploratory questionnaires were not continued due to ceasing active case follow-up for the outbreak at the end of July 2022. Gathering exposure data over a long period of interest (3 months) covered in the interviews was likely a limiting factor in the ability to gather accurate exposure information related to illness. Therefore, the results of the exploratory interviews (and incorporated in the tables above and below) must be interpreted in light of insufficient interviews having been completed to fully inform hypothesis generation related to a food or environmental exposure association with illness using an iterative exploratory based interview approach.

5.4.9 Thresholds

Figures 6a and 6b below shows all the food, drink and water exposures identified as meeting the thresholds (although they may not be considered high consumption) as defined in the

methods section. These items exceeding 20% in the questionnaires, or 50% in the interviews are also shown below.

Figure 6a. Consumption rates from questionnaires and exploratory interviews over the pre-determined thresholds set (>20% and >50% respectively)

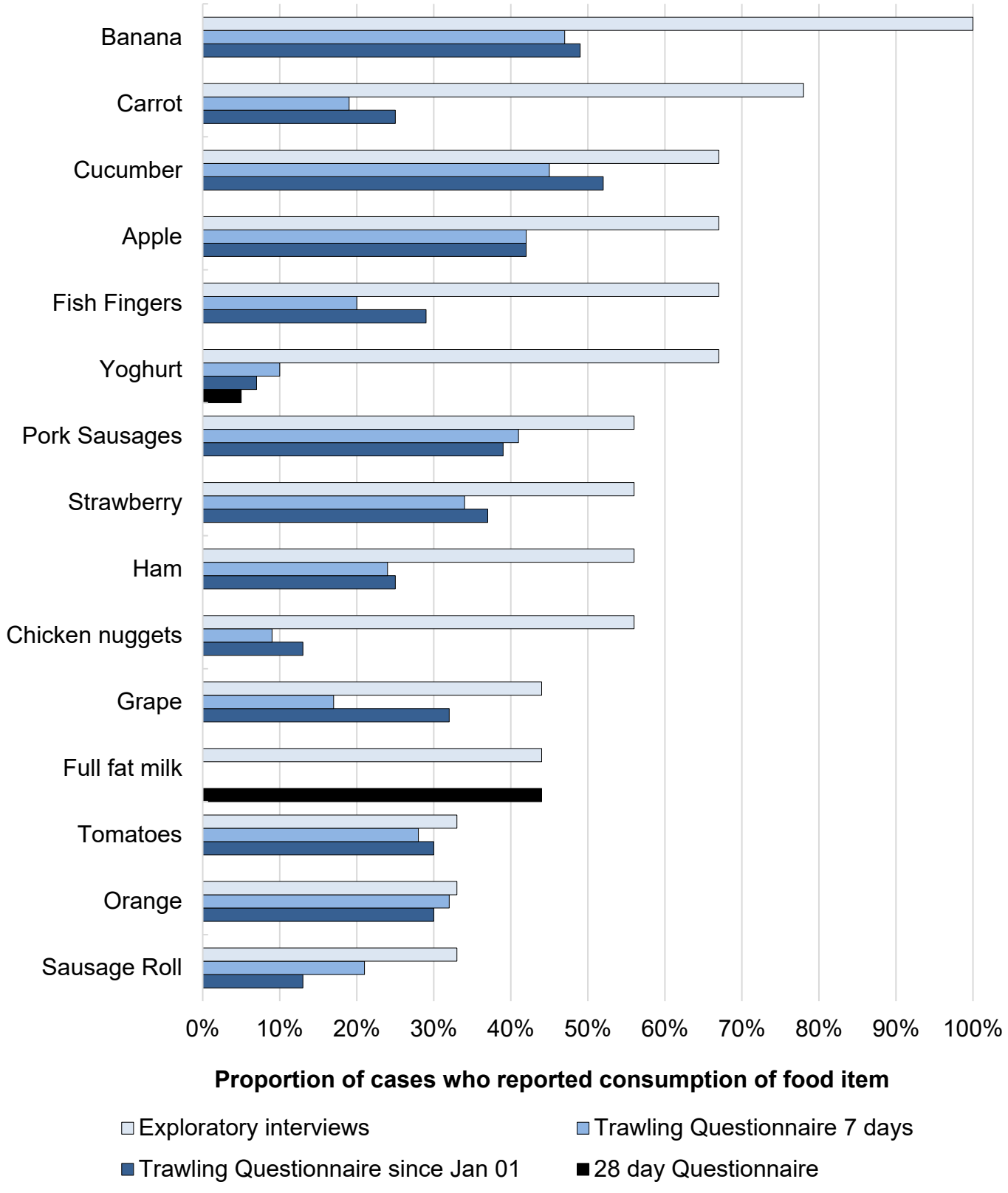
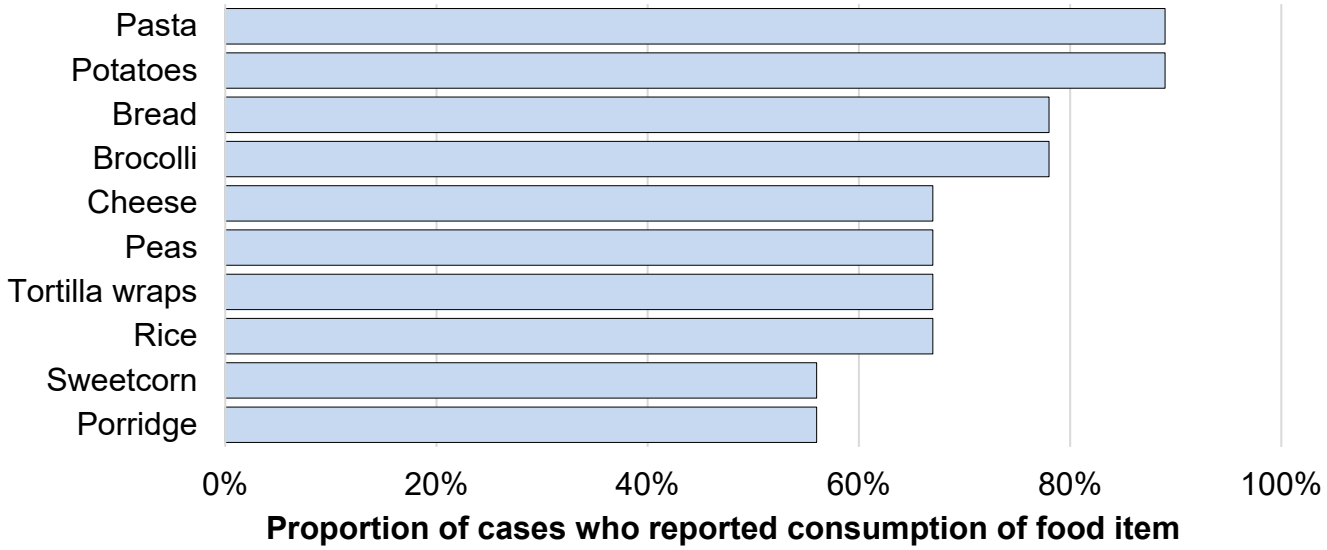


Figure 6b. Consumption rates of food items identified from exploratory interviews only over the pre-determined thresholds, >50%



5.4.10 Comparison

Table 18 and Figure 7 show that there are a number of food items, where consumption rates shown in Figures 6a and 6b are reported at a different rate to that expected based on the data available from the 2 comparator data sources identified. This comparison highlights the items that may need further consideration and exploration as to a potential causes or contributory exposures of hepatitis.

Figure 7. Comparison of reported case consumption rates from questionnaires and exploratory interviews to National Diet and Nutrition Survey (NDNS) and market research panel (MRP) consumption rates

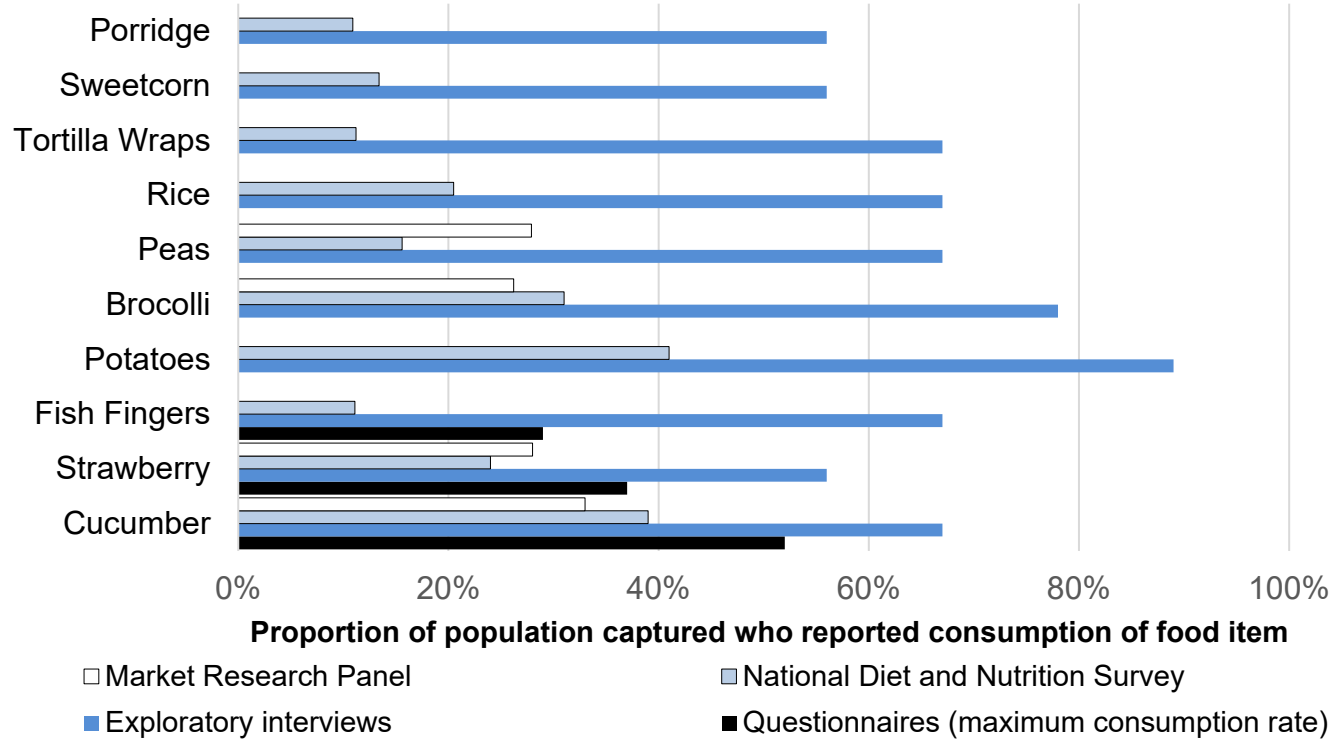


Table 18. Food, drink and water exposure items identified as meeting the defined thresholds in the trawling questionnaire or exploratory interviews, compared to national available data

Exposure	Trawling Q: Since 1 January[1] (consumption >20% or >5% and unexpected)	Trawling Q: In 7 days before onset of illness (consumption >20% or >5% and unexpected)	28-day Q: In 2 months before onset of illness	Interviews: In 3-months before onset of illness (consumption >5 (>50%))	Comparator (NDNS): consumption over 4-days[2]	MRP: consumption over 7 days[3]
Full fat milk	-	-	14 (44)*	<5	25.8%	
Dairy or lactose free diet	7 (12)*	-	<5	<5		2.7%
Cucumber	29 (52)*	14 (45)*	-	≥5*	39 %	32.8%
Tomatoes	17 (30)*	9 (28)*	-	<5	7.4%	40.4%
Potatoes	<5	<5	<5	≥5*	41%	
Peas	<5	-	-	≥5*	15.6%	27.9%
Broccoli	<5	-	-	≥5*	31%	26.2%
Sweetcorn	<5	<5	-	≥5*	13.4%	
Fish fingers	19 (29)*	7 (20)*	-	≥5*	11.1%	
Chicken nuggets	8 (13)	<5	-	≥5*	5.8%	24.6%
Sausage	24 (39)*	14 (41)*	-	≥5*	31.1%	
Sausage roll	8 (13)	7 (21)*	-	<5	11.4%	
Ham	15 (25)	8 (24)	-	≥5*	37.6%	
Gammon	7 (11)	6 (18)	-	≥5*	2.3%	

Exposure	Trawling Q: Since 1 January[1] (consumption >20% or >5% and unexpected)	Trawling Q: In 7 days before onset of illness (consumption >20% or >5% and unexpected)	28-day Q: In 2 months before onset of illness	Interviews: In 3-months before onset of illness (consumption >5 (>50%))	Comparator (NDNS): consumption over 4-days[2]	MRP: consumption over 7 days[3]
Beef mince	11 (18)	6 (17)	-	≥5*	17.6%	
Rice	<5	-	-	≥5*	20.5%	
Pasta	6*	<5	<5	≥5*	47.5%	
Porridge	<5	-	-	≥5*	~10.9%	
Tortilla wraps	-	-	-	≥5*	11.2%	
Grape	21 (32)*	15 (39)*	-	<5	42%	29%
Banana	32 (49)*	18 (47)*	-	≥5*	60%	59%
Strawberry	23 (37)*	13 (34)*	-	≥5*	24%	27.9%
Brand B sweets	19 (53)*	6 (38)	-	<5	14.1%	
Brand C chocolate	23 (40)*	10 (34)	-	<5	4%	

[1] A number is provided where the value is >5, or where the denominator is not available (because it is based on comments not responses to a question), or a percentage cannot be calculated.

[2] NDNS data estimates typical consumption of foods in the general population over 4-days. Underreporting is a known approximated at around 29% lower than typical consumption within the UK. NDNS data was split by age group and consumption averaged across the 1.5 to 10 year age group. The most comparable food type was used.

[3] Market Research Panel (MRP) data was collected from 183 control questionnaires to inform the analytical study of an outbreak of Salmonella. Application of data collected for another outbreak may impact how data can be structured and stratified. Those responses from the 15% completed for 0 to 5 year olds (slightly younger than our case range, were used, where a question was specifically asked of a control, to inform this average. This was taken over a 7-day time-period. Other limitations include the representativeness of those who complete MRPs, data quality concerns, coverage bias and non-response.

From this table we can see that consumption of some food items as well as meeting our pre-determined thresholds was >10% above a comparator group. These food items include:

- cucumber
- tomato
- potato
- peas
- broccoli
- sweetcorn
- fish fingers
- rice
- porridge
- tortilla wraps
- banana
- strawberry
- brand C chocolate

Brand C chocolate were a specific prompted question and this was not seen for chocolate items in general, so this finding is likely to have been influenced by another outbreak investigation occurring at the same time.

5.4.12 Comparative analysis

We had MRP data available on cucumber, tomatoes, peas, broccoli, banana and strawberry consumption.

We therefore compared the items using a 2 proportions z-test. The results are presented in Table 19 below.

Data on the population makeup (denominator) used for the NDNS results was not available and therefore it was not possible to complete a 2 proportions test with this comparator group.

Table 19. Results of the 2 proportions z-test comparing food consumption reported by cases to consumption in the MRP population

Food consumed (exposure)	Rate of consumption in market research panel (MRP) [95% CI]	Highest rate of consumption from trawling questionnaire [95% CI]	Test of proportion of MRP to trawling questionnaire rate p value	Highest rate of consumption from exploratory interviews [95% CI]	Test of proportion of MRP to exploratory interview rate p value
Cucumber	0.33* [0.16-0.54]	0.52* [0.38-0.65]	0.1*	0.67* [0.30-0.93]	0.07*
Tomatoes	0.40 [0.22-0.61]	0.3 [0.18-0.43]	0.35	0.33 [0.07-0.70]	0.7
Peas	0.28 [0.14-0.50]			0.67 [0.30-0.93]	0.04*
Broccoli	0.26 [0.11-0.46]			0.78 [0.40-0.97]	0.006*
Banana	0.59 [0.39-0.78]	0.49 [0.37-0.62]	0.38	1 [0.66-1]	0.02*
Strawberry	0.28 [0.14-0.50]	0.37 [0.25-0.50]	0.46	0.56 [0.21-0.86]	0.13

* An asterisk indicates those items with a p value following the 2-proportion z-test of <0.1.

Cucumber is the only food item when compared to the available MRP data that may be consumed differently by cases when compared to the MRP population (p=0.1). However, when comparing the MRP data and exploratory interviews some other food items are of interest – peas, broccoli and bananas. It should be noted that the data for these tests involve different methods of data collection. Other foods of interest, not comparable on the MRP remain as:

- potato
- sweetcorn
- fish fingers
- rice
- porridge
- tortilla wraps

6. Discussion

6.1 Epidemiological discussion

This report collates together several sources of information to explore the possible contribution of a food or environmental exposure driving the recent increase in acute hepatitis in children. However, the sources of data included in this report each collect data in a different and limited way. The content is rich, but the design of the trawling questionnaire and the 28-day questionnaire limited the analysis or in particular instances it was not easy to verify and compare with the other sources of data on case exposures. The quality of data limited the analysis, and this was further limited by the time available for in depth analysis, including consideration of other exposures and progression of hypothesis generation through an exploratory based case interview approach. Pharmaceutical and pet-based exposures and timelines from symptom onset to outcome have not been reviewed. Conclusions drawn must therefore bear in mind that this is limited analysis and other exposures of interest may yet be identified.

What the findings do show is that amongst the 77 cases included in the de-duplicated trawling questionnaire and the 38 new cases in the 28-day questionnaire data lists, there is a mix of male and female cases, and most are aged between 2 and 5 years old. The age of cases is of public health significance both in understanding the long-term health impacts of this outbreak and illness on these children and costs to the NHS, but also epidemiologically – noting that parents completing trawling questionnaires and exploratory interviews (whose children in these age groups attend nursery or other childcare settings) may not know all the foods consumed in the childcare setting. Likewise, remembering food consumed back to 1 January 2022 for the trawling questionnaire, or back 2 to 3 months for those responding to the 28-day questionnaire and exploratory interviews respectively, have a significant risk of recall bias – with more commonly and recently eaten foods more likely to be remembered than ‘one-offs’ and less commonly consumed foods, specific dishes and meals or snack foods or the possibility of so called ‘stealth’ vehicles. For example constituent ingredients in other dishes.

It should also be noted that underreporting is a known issue in NDNS as well as other dietary surveys, approximated at around 29% lower than typical consumption within the UK NDNS. This is also true when asking those responding to the 28-day questionnaire about the exposures in the 2-months prior to symptom onset. Therefore, underreporting is likely to be an issue in both the questionnaire responses and comparator survey data.

The quality of data limited the ability to derive meaningful interpretation of findings. For the trawling questionnaire, coding of data on exposures to drink and food items meant reported consumption needed to be drawn out of free text from responses. In some cases, this was from a single variable, but in others from multiple variables as the questions asked about multiple food types. For example:

1. Exposures to fruit-based drinks may not have been explicitly disclosed as this was not how Q13d was phrased “Consumption of fruit (dried, canned, fresh, frozen, juice, smoothies)”. Individuals may have responded about fruit, or about drinks, or about both – and without knowing if questionnaires specifically asked about each it is hard to know what is meant by a yes response or by omissions in the description.
2. Some exposures such as cucumbers and carrots are recorded in multiple places. Likewise, sausage rolls were identified as desserts by 3 respondents and not as a source of meat. This demonstrates the subjectivity of interviewers and respondent’s categorisation of foods into certain food groups, which may not be applied consistently across interviews. This shows the confusion where cases report consumption and highlights the risk that using unprompted free text may have had in underreporting and recording.
3. In the questionnaire design savoury pastry and bread items were included in dessert-based prompts, and cakes and biscuits as a pudding item not as snacks. Biscuits were not separated as biscuit or chocolate-based items making analysis in the correct food groups difficult – especially when no free text was included.

These points emphasize the requirement for careful questionnaire design, involving experts from all relevant subject areas and interviewer training to ensure consistency and harmonisation of data collection to ensure as far as possible that the inherent complications in gathering exposure information from cases or parents can be mitigated as far as possible and robust analysable data is obtained for epidemiological studies.

Exposures identified as more than 20%, or with a change of more than 50% of consumption between the period since 1 January 2022 and in the 7 days before illness onset, or more than 5% and unexpected in this age group are still considered to warrant further investigation.

For cucumbers (as an example of the most common salad item consumed, and the food item which the highest proportion (52%) of trawling questionnaire respondents reported exposure to), there has been a documented previous outbreak of gastrointestinal illness associated with cucumber exposure and toxicological contamination (6), however cases presented differently. We therefore need to explore carefully whether cucumber is a plausible causative salad item in a large, relatively long duration outbreak. Depending upon the international distribution of the cases, the time of the year, known trade patterns and seasonally affected human behaviours (for example, increased salad consumption during summer months when picnics and barbeques may be more common) then items such as cucumbers could explain this distribution. Historical experiences of salad-based outbreaks is that they usually cause short duration outbreaks due to the short shelf life of the product, with persistent contamination at source rarely resulting in long duration outbreaks. On the other hand, new sources of cucumber contamination and changes in the patterns of production, storage and distribution of this particular crop in recent years may have occurred that could explain cases arising over a period of weeks and months, with changes in trade patterns also a consideration post EU Exit.

It may also be that the salad items such as cucumbers, carrots and tomatoes (other commonly consumed items) are a confounder for another food item, for example, a mixed salad with other

‘stealth’ constituents such as sprouted seeds, burger or a dip or sauce as they are often consumed together. Cucumbers and tomatoes are also 2 of the items consumed by a higher proportion of cases than would be expected.

Houmous may be a common dip for crudites (typically carrot, cucumber and pepper sticks) for children of this age. It is also plausible that mould could be associated with humous. Although houmous consumption was asked about in the exploratory interviews less than 5 cases reportedly consumed this item, and there was no mention of it being old, out-of-date or mouldy. Consumption of pork products was also higher than any other individual meat based item, this was particularly true for sausages and ham. For sausage rolls a proportional increase was also seen in consumption since 1 January 2022 and in the 7 days before onset of illness. However, consumption of these products against national comparators highlighted that these are commonly consumed by children of this age and the rates were not largely unexpected.

Consumption of fish fingers was reported as 29% in the trawling questionnaire and 67% in the exploratory interviews. This is higher than in the NDNS at 11.1%. Reported strawberry, apple and banana consumption was all above 20% in the trawling questionnaire, and likewise in the exploratory interviews, all respondents eating bananas, and more than 5 apples and strawberries. This level of reported consumption appeared as expected for apples, with strawberries and bananas slightly higher than expected – depending upon the data source compared to.

Reported consumption of Brand C chocolate was higher than would be expected. This needs to be considered in the context of a recent national food recall and the impact on recall bias, and of the IMT request to specifically prompt this question during the trawling questionnaire interviews.

The way that the trawling questions were asked, with no specific prompting (with the exception of Brand C chocolate of types of food and with many of the food groups we are most interested in (for example corns, wheats or maize) not included in the trawling questionnaire, we cannot rule in or out some of the potential hypothesis for associated exposures. More than 50% children consumed wheat bread, but again of different types and brands. Porridge is identified by more than half exploratory interview respondents although a mixture of brands identified. Consumption of pasta and rice was also more than 50% of respondents, and corn-based tortilla wraps were consumed by more than half the respondents to the exploratory questionnaires. Sweetcorn was also identified as an item eaten by more than half of cases in the interviews. These exposures had not been identified from previous data sources. Consumption of all these food items was higher than expected based on the comparators used.

From the data available so far from the trawling questionnaire, the exploratory interviews, and rapid literature reviews, food exposures of greatest interest for further exploration include:

- any dips or sauces such as hummus used with crudites or salad
- any cucumber, tomato, potato, broccoli, pea or sweetcorn consumption

- any strawberry or banana consumption
- any fish finger consumption
- any corn, wheat, maize or oat consumption including porridge oats and tortilla wraps
- (possibly) pork and chicken consumption, in particular ham, sausages, sausage rolls and chicken nuggets

Although the number of children consuming dairy free products appeared higher than would be expected in the trawling questionnaire this was not found in the 28-day questionnaire or exploratory interviews. These rates compared with nationally expected ones.

From a public health perspective, it is important to consider that there may be alternate or contributory causes to an outbreak, especially when the aetiology is unknown. For outbreaks and incidents of unknown aetiology the UKHSA cluster guidance should be used (27). This descriptive epidemiology summary thus far shows that there are a number of possible environmental or food exposures which could be associated with hepatitis. This should be considered and explored in more detail with further descriptive and subsequent analytical epidemiological studies to identify if hepatitis of unknown origin is associated with an environmental/food or as yet undefined or unexplored exposure to ensure that public health recommendations can be made to reduce exposure to that item. The potential severity of outcomes for these children both in the short and long-term adds to the weight and importance of a thorough investigation of all potential exposures.

6.2 Discussion summary

The analysis of food, drink and water exposures from these 4 sources of data has been completed to 29 July 2022. This has identified 33 items where consumption exceeded a threshold set for inclusion of exposures of interest. When considered alongside national comparators for consumption we see that 15 items have higher than expected consumption recorded in at least one information source. Some of these may be explained by reporting biases between interviews and surveys, and the methodology used for data collation. For example Brand C chocolate are potentially over-reported compared to other food items due to specific prompts for this particular product in the trawling questionnaire. However, the conclusions derived from this descriptive analysis are those exposures that require further exploration which include fruits (bananas and strawberries), vegetables and salad items (tomatoes, sweetcorn and cucumbers), fish fingers and tortilla wraps. Bananas are reported by all 9 of the recently interviewed cases where the exploratory interview approach was used and are also the most reported fruit consumed in the trawling questionnaire data but reported consumption is generally consistent with previous market research panel data and NDNS data for children of a similar age. A possibly higher than expected frequency of reported cucumber consumption is flagged as it is higher than any other vegetable or salad item consumption and higher than market research panel and NDNS derived consumption data.

The format of the trawling questionnaire made it hard to identify clear common exposures with sufficient granularity of detail. Open questions about dietary items/food groups are likely to under-detect specific foods. The 28-day questionnaire asked more specific closed questions, but items/exposures covered were limited, and part way through the process the questionnaire was modified to ask respondents if they would be willing to take part in a more in-depth food questionnaire and therefore data on food-exposures was not always collected. It is therefore suggested that questions on these and other key food items are added to the standard questionnaire in any planned case-control study, based on the outcomes of this descriptive analysis. The importance of multi-disciplinary engagement in the study design, including questionnaire design, needs to be initiated at the very start to ensure the scale, utility and quality of the data collected for analytical epidemiological studies is maximised. Where sufficient weight of evidence is obtained to ensure targeting of efforts and resource, food samples should be examined for traces of contaminants, including pesticides, mycotoxins or viral and other pathogens relevant to a plausible explanation of any identified potential associations. Inclusion of other data sources such as trade data and supply chain information, while time consuming to collate and complex in some circumstances to analyse would also help inform hypothesis generation.

It is also important to note that this investigation has highlighted the importance of a One Health approach and the use of cluster guidance and multi-disciplinary teams in investigating gastrointestinal outbreaks and incidents of unknown aetiology (27). This has been recognised in more recent incidents. Likewise, UKHSA Toxicology Group now has the capability to test for new toxins including mycotoxins where the epidemiological hypothesis warrants this.

7. Conclusions

1. Reported consumption of some vegetables, fruit, salad items, fish, meat, sweets, dairy, rice, wheat and corn products for cases was over 20%. Consumption of some items appears to be higher than would be expected in this age group. This includes cucumbers, bananas, strawberries, sweetcorn, tomatoes, fish fingers and tortilla wraps, but recall accuracy have to be considered. These preliminary findings need to be interpretated with caution. A comparison with frequency of reporting in national surveys of food consumption in children of similar age points to some possible excess frequency of consumption for a few specific food items, but the lack of comparability between selection of cases and a control group set to the same time period, covering questions related to the same food items or exposures of interest and seasonal period, means the apparently higher than expected reported consumption of some food items requires further investigation before it can be confirmed.

2. There is a plausible biological pathway for pesticides or other food toxins such as mycotoxins to cause or contribute (alongside a viral causative pathogen) to hepatitis in children.

3. The international distribution of cases (if true exceedances in all countries reporting cases) and duration of the outbreak is less coherent with this hypothesis. A limitation of any conclusion based on crop and trade patterns is that a country identified by trade patterns may not be the country of origin of the food item and contaminants present in it.

4. UKHSA has already produced guidance for investigation of clusters of illness with an unknown cause, including possible infectious and non-infectious causes Non-infectious disease clusters: investigation guidelines (27). The application of such guidance to this incident indicates that overall, work on any exposure that could represent an explanation of this cluster of hepatitis with unknown cause, appeared to lack coordination. Guidance on management of clusters of unknown illness appeared to have been only partially implemented at the start of this outbreak. Activities to scope and then examine the literature, the descriptive epidemiology of exposures ranging from infectious to non-infectious alongside microbiological and toxicological laboratory findings should be included from the start in future incidents.

8. Recommendations

Based on these limited literature reviews, lab tests and descriptive epidemiology findings so far, we recommend:

1. Given the severity of hepatitis identified in the UK and possibly elsewhere, UKHSA should consider establishing an ongoing surveillance scheme for non-A-non-E hepatitis with a view of collating information on cases over several years, for analyses and identification of potential causes (exposures that include viral and other infectious as well as non-viral causes such as chemicals) over an appropriate time period. Such surveillance would include several steps and activities to be developed and agreed, including wide consultation with UKHSA specialist teams and divisions and potentially relevant agencies such as FSA, APHA and DEFRA, and would benefit from inclusion of plans for biological and environmental (food and other) sampling as part of an overall design and set of activities.

2. Based on literature reviews conducted, there is plausibility for chemical causes or contributory causes of this incident. Findings so far have highlighted a few specific food items that may act as vehicle for a causative exposure. A further analytical study should be designed on plausible non-viral causes or contributory factors including environmental exposures should be incorporated into existing virological case-control studies.

3. UKHSA procedures for investigation of a cluster of illness in the absence of a known cause or combination of causes may benefit from a review, with the goal of ensuring that several scientific lines of investigation benefit from an approach that is co-ordinated at a strategic level. It is likely that UKHSA procedures on this would benefit from involvement of specialists from a range of disciplines early in the IMT and in the development of a trawling questionnaires where the exposure causing illness is unknown – either in advance using existing templates, or as a specific collaborative questionnaire in response to an incident.

4. If cases of non A-E hepatitis start to increase again, more descriptive questions on the consumption of these food items identified and other environmental exposures should be included in the questionnaire and incorporated in further analytical study design and execution. This should include the frequency and dose, brands, and product handling including washing, peeling and cooking procedures.

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Appendix 1. Rapid literature review search strategy

Search term concepts and combinations 1: hepatitis/liver terms

1. exp hepatitis/
2. exp chronic hepatitis/
3. exp virus hepatitis/
4. exp liver toxicity/
5. exp liver injury/
6. exp toxic hepatitis/
7. exp liver failure/
8. exp liver abscess/
9. exp end stage liver disease/
10. exp acute on chronic liver failure/
11. exp liver disease/
12. exp liver cirrhosis/
13. exp acute liver failure/
14. exp liver circulation/
15. exp liver vein/
16. exp fatty liver/
17. exp jaundice/
18. ((acute or chronic or fulminat* or fulminant) adj3 hepatitis).tw,kw,kf.
19. ((acute or chronic or fulminat* or fulminant) adj3 (liver or hepatic) adj3 (fail* or necro* or disease* or dysfunction or injur* or function*)).tw,kw,kf.
20. toxic hepatitis.tw,kw,kf.
21. hepatic immune function.tw,kw,kf.
22. or/1-21

Search term concepts and combinations 2: child terms

23. exp child/
24. exp child health/
25. exp child health care/
26. exp hospitalized child/
27. exp infant/
28. exp newborn/
29. exp infant welfare/
30. exp infant care/
31. exp newborn disease/
32. exp newborn intensive care/
33. exp adolescent/
34. exp adolescent health/

35. exp hospitalized adolescent/
36. exp pediatrics/
37. exp pediatric emergency medicine/
38. (infan* or newborn* or new-born* or neo-nat* or neonat* or picu* or nicu* or baby or babies or toddler* or child* or adolescen* or pediatric* or paediatric* or preschool* or pre-school* or boy* or girl* or kinder or kindergart* or kid or kids or enfant* or nursery or nurseries or creche* or daycare* or youth or youngster* or juvenil* or student* or pupil* or teen* or pubesc* prepubesc*).tw,kw,kf.
39. or/23-38

Search term concepts and combinations 3: adenovirus terms

40. exp Adenoviridae/
41. exp adenovirus infection/
42. (adenovirus or adenoviridae).tw,kw,kf.
43. or/40-42

Search term concepts and combinations 4: mycotoxin terms

44. exp mycotoxin/
45. exp masked mycotoxin/
46. exp ergotism/
47. exp aflatoxin/
48. exp ochratoxin/
49. exp fumonisin/
50. exp citrinin/
51. exp patulin/
52. exp sterigmatocystin/
53. exp zearalenone/
54. exp T 2 toxin/
55. exp Aspergillus/
56. exp Fusarium/
57. mycotoxin*.tw,kw,kf.
58. ergotism.tw,kw,kf.
59. (mold or molds or mould or moulds).tw,kw,kf.
60. aflatoxin*.tw,kw,kf.
61. ochratoxin*.tw,kw,kf.
62. fumonisin*.tw,kw,kf.
63. citrinin.tw,kw,kf.
64. patulin.tw,kw,kf.
65. sterigmatocystin.tw,kw,kf.
66. zearalenone.tw,kw,kf.
67. T-2 toxin*.tw,kw,kf.
68. aspergillus.tw,kw,kf.
69. fusarium.tw,kw,kf.

70. cyclopiazonic acid.tw,kw,kf.
71. HT-2 toxin.tw,kw,kf.
72. deoxynivalenol.tw,kw,kf.
73. nivalenol.tw,kw,kf.
74. or/44-73

Search term concepts and combinations 5: other viruses/contaminants

75. exp virus/
76. exp viremia/
77. exp infectious agent/
78. exp bacterium/
79. exp severe acute respiratory syndrome coronavirus 2/
80. exp coronavirus disease 2019/
81. exp experimental coronavirus disease 2019/
82. exp herpes virus infection/
83. exp food contamination/
84. exp pesticide/
85. exp herbicide/
86. exp fungicide/
87. virus*.ti,kw,kf.
88. viremia.tw,kw,kf.
89. pathogen*.ti,kw,kf.
90. bacteri*.ti,kw,kf.
91. (coronavirus* or 2019nCoV* or 19nCoV* or "2019 novel*" or Ncov* or "n-cov" or "SARS-CoV-2*" or "SARSCoV-2*" or SARSCoV2* or "SARS-CoV2*" or "severe acute respiratory syndrome*" or COVID*2).tw,kw,kf.
92. herpes.tw,kw,kf.
93. contamin*.tw,kw,kf.
94. pesticide*.tw,kw,kf.
95. herbicide*.tw,kw,kf.
96. fungicide*.tw,kw,kf.
97. ((chemical or environmental) adj cause*).tw,kw,kf.
98. or/75-97

Excluded cancer terms

99. exp malignant neoplasm/
100. exp leukemia/
101. exp carcinoma/
102. neoplasm*.ti.
103. leuk?emia.ti.
104. carcinoma*.ti.
105. cancer*.ti.

106. (tumor or tumour*).ti.
107. or/99-106

Search combinations*

Search 1: Joint effect of adenovirus and mycotoxins: concepts 1 and 2 and 3 and 4

108. 22 and 39 and 43 and 74
109. 108 not 107
110. limit 109 to English language

Search 2: Effect of mycotoxins alone on liver function (hepatitis): concepts 1 and 2 and 4

111. 22 and 39 and 74
112. 111 not 107
113. limit 112 to English language

Search 3: Joint effect of mycotoxins with other contaminants/viruses: concepts 1 and 2 and 4 and 5

114. 22 and 39 and 74 and 98
115. 114 not 107
116. limit 115 to English language

Search 4: Joint effect of adenovirus and any other contaminant/virus: concepts 1 and 2 and 3 and 5

117. 22 and 39 and 43 and 98
118. 117 not 107
119. limit 118 to English language

* The following terms were also excluded: ‘Transplant’ and ‘Fungus and mushroom-related terms’.

List of abbreviations

Abbreviation	Meaning
AAV2	Adeno-associated virus 2
ALP	Alkaline phosphate
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
COT	Cyclopiazonic acid
GC/MS	Gas chromatography-mass spectrometry
GC-MS/MS	Gas-chromatography tandem mass spectrometry
IARC	International Agency for Research on Cancer
ICPMS	Inductively coupled plasma mass spectrometry
IMT	Incident Management Team
IU/l	International Units/Litre
LC-HRMS	Liquid chromatography-high resolution orbitrap mass spectrometry
MDA	Malondialdehyde
NDNS	National Dietary and Nutrition Survey
NICC	National Incident Co-Ordination Centre
SGSS	Second Generation Surveillance System
UKHSA	United Kingdom Health Security Agency

Environmental exposures investigation team

In alphabetical order of surname:

Name	Department
Rebecca Close	Environmental Epidemiology
Helen Crabbe	Environmental Epidemiology
Tony Fletcher	Environmental Epidemiology
Kerry Foxall	Toxicology
Britta Gadeberg	Toxicology
Gauri Godbole	Gastrointestinal Infections and Food Safety (One Health)
Ann Hoban	Gastrointestinal Infections and Food Safety (One Health)
Neelam Iqbal	Environmental Epidemiology
Lesley Larkin (joint coordination)	Gastrointestinal Infections and Food Safety (One Health)
Giovanni Leonardi (joint-coordination)	Environmental Epidemiology
Tim Marczylo	Toxicology
Ovnair Sepai	Toxicology
Hannah Taylor	FETP, Environmental Epidemiology

About the UK Health Security Agency

UKHSA is responsible for protecting every member of every community from the impact of infectious diseases, chemical, biological, radiological and nuclear incidents and other health threats. We provide intellectual, scientific and operational leadership at national and local level, as well as on the global stage, to make the nation health secure.

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For queries relating to this document, please contact: acutehepkids@ukhsa.gov.uk

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