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Laboratory investigations into the cause of multiple serious and fatal food poisoning incidents in Uganda during 2019

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Title: Laboratory investigations into the cause of multiple serious and fatal food poisoning incidents in Uganda during 2019

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Highlights

- International response to food poisoning incidents
- Array of analytical methodologies used in investigation
- Tropane alkaloids implicated as source
- All outbreaks linked to one source of contaminated food

Abstract

During 2019 a series of food poisoning events and deaths occurred in Uganda due to an unknown causative agent. In response the World Food Programme (WFP) initiated investigations into the incidents which included contacting expert laboratories to analyse the main suspect product known as Super Cereal. The laboratories used a broad gamut of their testing capabilities to determine the cause of the incidents. This included targeted and non-targeted methodologies such as LC-MS/MS, QToF-MS and spectroscopic techniques coupled to chemometrics. The conclusion of the investigation was that the cause of the food poisoning was due to the presence of the Tropane alkaloids, atropine and scopolamine, in the Super Cereal. These alkaloids were found to be present due to the incorporation of Datura seeds during production of the Super Cereal. Furthermore, the data generated showed all outbreaks were caused by a single batch of the Super Cereal.

Keywords

Food Poisoning, Tropane Alkaloids, Atropine, Scopolamine.

1. Introduction

Context of the Super Cereal Incident in Uganda

This paper outlines the laboratory investigation into a number of serious food poisoning episodes which occurred in Uganda in 2019. This was in response to a World Food Programme (WFP) request to several expert laboratories to support their own investigations into the issues. In all the incidents recorded a product known as 'Super Cereal' was suspected of causing the illnesses and this paper outlines steps taken to investigate this product and identify the causative agents.

In terms of the sequence of events, between the 13th and 20th March 2019 a total of 278 cases of food related illness were reported in the Amudat and Napak districts of the Karamoja Region of Uganda. Five fatalities were recorded as being caused by the illness and the remaining 273 affected individuals made a full recovery. Nine new cases were reported on the 9th April, and all that fell ill recovered. These appeared to be linked to the first episode as all had consumed the same suspect batch of Super Cereal (SC) food, which had been stored away since the March incident. In a third, and seemingly different incident, 33 cases of food poisoning were reported in the Lamwo District of Uganda, some 400 km from Amudat between the 28th and 29th August. However, again SC was suspected of causing the issues, but this material was from a completely different supply chain which was extremely worrying. While two of the cases were recorded as being severe no fatalities were recorded and all made a full recovery. No clear link between the first and third incidents could be established at that time. The potential to have to cease all supplies of Super Cereal into Africa was a real risk.

In all three incidents the symptoms recorded were very similar. A rapid onset (one to two hours) after consumption of SC, patients presented with a range of symptoms including – headache, abdominal pain, dry mouths, sticky saliva, body itching, mental confusion dizziness, paleness and dilated pupils.

A number of UN Agencies (World Health Organization, WFP) and the Ministry of Health in Uganda supported by the Center for Disease Control (CDC) in the United States were active in investigating the cause of the outbreaks (Flynn & Beach, 2019). In this paper the actions taken by the WFP supported by scientific evidence provided by the Institute for Global Food Security, Queen's University, Belfast UK, Merieux Nutrisciences, and the Center for Food Safety and Applied Nutrition, United States Food and Drug

Administration, USA are detailed (Croley, Knolhoff, O' Donnell, Mangrum, Fong Sam, Callahan, & Henderson, 2020).

Actions taken by WFP following the initial reports of illness

Between the 13th and 15th March WFP suspended the distribution of SC in the affected areas. On the 16th March WFP stopped distribution of SC across the country as a precautionary measure. The Ugandan Government, Police and WFP collected samples of SC from the affected districts. There were no reports of any anomaly observed in any of the samples. On the 17th March the WFP initiated the retrieval of SC stocks from affected areas. These were held by the UN and local police. At the same time a community engagement plan was launched to inform the local population. Samples were dispatched to a number of laboratories for analysis. Preliminary microbiological testing did not show any evidence of cause. Further test data was made available by WFP on the 26th March and this indicated no evidence of pesticides, mycotoxins or heavy metal contamination of SC to be the cause of illness. Further testing of SC samples was undertaken independently using untargeted analysis at three laboratories. All laboratories reported that they had found the presence of several tropane alkaloids, such as atropine and scopolamine to be present at parts per million concentrations in tested samples, levels at which acute toxicity are highly likely to be exhibited. Based on the lack of any other potential causative agents being found, and the symptoms of those who became ill after consumption that had many features associated with alkaloid poisoning, this was concluded to be the most likely cause of the first two outbreaks(Whitworth, 2019). However, while no link between these incidents and the third outbreak had been established, it was recognized that the same toxic agents were the likely cause. This paper concentrates on the analysis of samples taken during the first and third incidents.

Tracking the source of contaminated Super Cereal

The supply chains for SC are complex but this is common with many food products that are processed and transported across national boundaries. Based on the lot numbers of the contaminated SC (2018EX017, 2018EX011, 2018EX025) the supplier was identified as a Turkish company. Products with the same batch number were shipped from a port in Turkey to Algeria, Tanzania and Kenya. The product in Kenya was dispatched to a number of other African countries, Uganda, Tanzania, Central African Republic, Rwanda

84 and Somalia. Along these supply chains there were a series of inspections of product and no issues in
85 terms of quality or safety were noted. In the product that reached Uganda, this was transported from a
86 central warehouse in Karamoja and onto other warehouses in Kotido, Kaabong and Moroto where further
87 distribution to around 90 health centres occurred. Again, during the in-country distribution no irregularities
88 were reported.

89 A technical inspection of the Turkish company was performed on or around the 17th April. Major systems
90 failures were recorded in quality management procedures. These have been detailed in a second report
91 on the Ugandan incidents (Abia, Nugent, Montgomery, & Elliott, 2020).

2. Materials and methods

2.1 Sample collection

Merieux Nutrisciences was mandated by WFP for the investigation of the 72 samples collected during the WFP sampling campaign following the 1st incident. These 72 samples were distributed in the following manner: 10 samples from retention stock in Turkey, 15 samples drawn from households in Uganda and 47 samples from storage warehouse in Uganda, Kenya and Somalia (Figure 1). Samples analysed (n=23) by QUB were provided by WFP via Merieux and included 11 of 15 suspect samples as well as 12 samples from retention stock in Turkey (Table 1).

Samples analysed by the FDA laboratory were obtained from a parallel investigation organized by the Ugandan government with the support of CDC. Seven suspect cereal samples were collected by the Ugandan Health Ministry from various local districts and three samples from retention stock were provided by WFP to be used as reference for analytical purpose.

Figure 1: Collection and distribution of SC samples for analysis by expert laboratories

Table 1: SC samples sent to QUB and FDA from 1st incident for laboratory analysis

<u>QUB Sample</u>	<u>Original Sample</u>	<u>Location Details</u>	<u>Lot</u>
<u>Code</u>	<u>Code</u>		
WFP-0001	MOR-24	SC Turkey returned from beneficiary	Unknown
WFP-0002	MOR-25	SC Turkey returned from beneficiary	Unknown

WFP-0003	MOR-26	SC Turkey returned from beneficiary	Unknown
WFP-0004	MOR-27	SC Turkey returned from beneficiary	Unknown
WFP-0005	MOR-29	SC Turkey returned from beneficiary	Unknown
WFP-0006	MOR-30	SC Turkey returned from beneficiary	Unknown
WFP-0007	MOR-31	SC Turkey returned from beneficiary	Unknown
WFP-0008	MOR-32	SC Turkey returned from beneficiary	Unknown
WFP-0009	MOR-33	SC Turkey returned from beneficiary	Unknown
WFP-0010	MOR-34	SC Turkey returned from beneficiary	Unknown
WFP-0011	MOR-35	SC Turkey returned from beneficiary	Unknown
WFP-0012	NG	Unknown Ikea bag	Unknown
WFP-0013	19-00005288	Dermirpolat, Turkey (SC reference sample)	494.5 MT-2.Lot 2019EX001
WFP-0014	19-00005284	Dermirpolat, Turkey (SC reference sample)	494.5 MT-2.Lot 2018EX032
WFP-0015	19-00005290	Dermirpolat, Turkey (SC reference sample)	516 MT-4.Lot 2019EX001
WFP-0016	19-00005286	Dermirpolat, Turkey (SC reference sample)	494.5 MT-3.Lot 2018EX032
WFP-0017	19-00005289	Dermirpolat, Turkey (SC reference sample)	494.5 MT-3.Lot 2019EX001
WFP-0018	19-00005285	Dermirpolat, Turkey (SC reference sample)	494.5 MT-2.Lot 2018EX032
WFP-0019	19-00005291	Dermirpolat, Turkey (SC reference sample)	516 MT-3.Lot 2018EX026
WFP-0020	19-00005281	Dermirpolat, Turkey (SC reference sample)	516 MT-3.Lot 2018EX026
WFP-0021	19-00005287	Dermirpolat, Turkey (SC reference sample)	516 MT-4.Lot 2018EX032
WFP-0022	19-00005282	Dermirpolat, Turkey (SC reference sample)	559 MT-10.Lot 2018EX031
WFP-0023	19-00005283	Dermirpolat, Turkey (SC reference sample)	430 MT-11.Lot 2018EX031
FDA-001	Sample A	SC from Kangole Napak	Unknown
FDA-002	Sample B	SC from Lorengecora Napak	Unknown
FDA-003	Sample C	SC from Lokopo District	Unknown
FDA-004	Sample D	SC from Amudat District	Unknown
FDA-005	Sample E	SC from Amudat District	Unknown
FDA-006	Sample F	SC from the warehouse	Unknown
FDA-007	Sample G	SC from the warehouse	Unknown
FDA-008	Sample 5 (19-00005292)	Dermirpolat, Turkey, (SC reference sample received directly from WFP)	494.5MT-3.Lot 2018EX032
FDA-009	Sample 6 (19-00005293)	Dermirpolat, Turkey, (SC reference sample received directly from WFP)	516MT-4.Lot 2018EX032
FDA-010	Sample 7 (19-00005294)	Dermirpolat, Turkey, (SC reference sample received directly from WFP)	494.5MT-2.Lot 2019EX001

2.2 Sample analysis

Samples were analysed using a variety of instrumental techniques in the three expert laboratories. The techniques included LC-HRMS, GC-HRMS, GC-MS, LC-MSMS, FTIR and NIR.

2.2.1 Sample preparation (QUB)

Sample (0.05g) was extracted in 1.5 mL of methanol/water solution (1:1, v/v), mixed for 10 minutes, sonicated for 15 minutes at maximum frequency in a water bath at room temperature, centrifuged at 10,000 g for 10 minutes at 4°C and the supernatant collected (1.0 mL). Subsequently, the extract was filtered through a 0.22 µm centrifuge tube filter (10,000 g at 4°C for 10 minutes). Filtered extracts were immediately transferred into maximum recovery vials for LC-HRMS analysis and a pooled sample acting as Quality Control (QC) was generated. Samples analyzed by the U.S. FDA were prepared in a generally similar fashion using water extraction and dilution with acetonitrile/1% formic acid (Croley et al., 2020).

2.2 Sample Preparation GC analysis (Merieux)

Cereal samples were prepared following an acid/base organic extraction protocol prior to GC-MS analysis. Distilled water was added to cereal samples then extracted with a mix of organic solvent with the first extraction carried out under acidic conditions then the second extraction conducted under basic conditions. Two organic extracts were then combined and concentrated under a flux of nitrogen. (Christen, Bieri, & Veuthey, 2007)

2.3 Sample Preparation LC and GC-HRMS analysis (Merieux)

Samples were extracted in acetonitrile/water solution, mixed for 10 minutes with a mechanical shaker, then water was removed by addition of anhydrous magnesium sulfate. Organic extract was transferred to vial and concentrated under reduce pressure before GC-HRMS analysis.

2.4 LC-HRMS analysis (QUB)

Analyses were carried out on an Agilent 1290 Infinity II LC system (Cheadle, UK) coupled to a 6545 QToF mass spectrometer (Santa Clara, CA, USA) equipped with a Dual Jet Stream electrospray ionisation source

operating in positive or negative mode with a dual nebuliser for real time accurate mass correction. Instrument settings were as follow: gas temperature was set at 240°C, drying gas flow at 10 L/min, nebulizer at 35 PSI, sheath gas temperature at 310°C, and sheath gas flow at 11 L/min. The capillary voltage was set at 3.5 kV in positive mode with a nozzle voltage of 300V and 2.5 kV in negative mode with a nozzle voltage of 700V, respectively. Fragmentor was set at 110V with a skimmer at 65V. Mass spectra data were acquired in continuum mode using All Ions methodology (low energy: 10 eV; medium energy: 20eV; high energy: 40 eV) over the range m/z 50-1200 with a scan time of 0.1 s. A reference mass solution containing a purine base and hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine) (HP-921) in ACN/water containing 0.1% formic acid (8:2, v/v) was continuously infused into the MS via the dual nebulizer.

The chromatographic separation was conducted on an Agilent Zorbax Eclipse Plus C18 column (50 mm x 2.1 mm, 1.8 µm). The column oven temperature was set at 45°C, injection volume at 5 µL and flow rate at 0.6mL/min. Mobile phase consisted of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid. The gradient was set as follows: 0.50 min of 10% (B) followed by a linear increase from 10 to 99% (B) over 7 min, isocratic cleaning step at 99% (B) for 1.5 min, then returned to initial conditions 1% (B) over 0.1 min and column equilibration step at 10% (B) for 0.9 min.

Prior to all analyses 10 QCs were injected in order to condition the system. For quality control pooled samples were injected at intervals of every 10 samples throughout the entire experiment to determine the chromatographic reproducibility of retention times and peak intensities.(Chatterjee, Chevallier, Wielogorska, Black, & Elliott, 2019)

2.5 LC-HRMS and MS/MS analysis (FDA)

LC-HRMS and MS/MS analysis at US FDA were performed as described in reference ((Croley et al., 2020)). Briefly, analyses were carried out on a Shimadzu Nexera LC system (Shimadzu Corp, Kyoto, Japan) coupled to a Thermo Q-Exactive mass spectrometer (ThermoFisher, San Antonio, CA,USA) equipped with a heated electrospray ionisation source operating in polarity switching mode. Mass spectra data were acquired in polarity switching mode at 70,000 resolving power for MS mode and separately in both positive and negative mode at 17,500 resolving power for MS/MS mode over the range m/z 100-1200. For data dependent acquisition, isolation window was set at 2Da, with a collision energy set at 40eV and an intensity

threshold of 8.3 E4 counts. The chromatographic separation was conducted on a Phenomenex Kinetex C18 column (150 mm x 2.1 mm, 100 Å, 1.7 µm) at 0.4 mL/min. Mobile phase consisted of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid.

2.6 GC-MS analysis (Merieux)

Analyses were carried out on an Agilent 7890B GC coupled to an Agilent 5975C Single Quadripole mass spectrometer operating in Electron Impact ionisation mode with MS Source and MS Quad set up at 230 °C and 150 °C, respectively. MS instrument was tuned using PFTBA per manufacturer recommendations. Data were acquired in SIM-SCAN mode from 35-450 AMU. The chromatographic separation was performed on an Agilent HP-5MS UI (30 m x 250 µm x 0.25 µm) column using Helium carrier as a carrier gas at a constant flow of 1.0 mL/min. Gradient was as follow: initial temperature of 60 °C was held for 5 minutes then ramped to 280 °C at a rate of 5 °C/min and held for 30 minutes, followed by return to 60 °C at a rate of 15 °C/min and held for a further 5 minutes at initial temperature. Samples were injected in splitless mode set at 280 °C using an Agilent straight liner with glass wool.

2.7 LC-HRMS and GC-HRMS analysis (Merieux)

GC analyses were carried out on a Thermo Scientific Q Exactive GC Orbitrap. Data were acquired in SIM-SCAN mode from 35-450 AMU. The chromatographic separation was performed on a Thermo Scientific TG-5SILMS (30 m x 250 µm x 0.25 µm) column using Helium carrier as a carrier gas at a constant flow of 1.5 mL/min. Gradient was as follow: initial temperature of 50 °C was held for 5 minutes then ramped to 320 °C at a rate of 15 °C/min and held for 20 minutes, followed by return to 50 °C. Data were analysed with TraceFinder 4.1 and Mass Frontier 7.0 (Thermo Fisher, US) and identification was based on NIST library. LC analyses were performed on Thermo Scientific Q Exactive LC Orbitrap using full scan mode from 70-1000 AMU. Chromatography was carried out using a Phenomenex Kinetex C18 column (3mm, 100 mm, 2.6 µm). Data were analysed using Mass Frontier 7.0, Compound Discoverer 2.1 (Thermo Fisher) and identification was based on library build with m/z Cloud (Thermo Fisher) and ChempSpider.

2.8 Spectroscopy Measurements

Fourier-transform infrared spectroscopy (FT-IR) and Near-infrared spectroscopy (NIR) spectra were collected on the multi-module iS50 spectrometer (ThermoFisher, Dublin, Ireland) at ambient temperature. The FT-IR samples were analysed on the benchtop FTIR with ATR accessory, diamond crystal and DLaTGS detector. Spectra were acquired in the 4000-400 cm^{-1} range with 32 scans and a resolution of 4 cm^{-1} . The NIR samples (5g) were poured onto the sample cup (diameter: 3.2 cm, height: 1.5 cm) spinner on the Integrating Sphere module of the instrument. Spectra were acquired in the 12000-4000 cm^{-1} range with 32 scans with a resolution of 8 cm^{-1} . Three replicates of each sample were measured and averaged prior to data pre-processing. (Haughey, Galvin-King, Ho, Bell, & Elliott, 2015)

2.9 Proximate Analysis by NIR

For benchtop NIR analysis, the spectra were recorded on an Antaris II FT-NIR (Thermo Fisher Scientific, Dublin, Ireland). The samples (10 g) were poured onto the sample cup spinner on the Integrating Sphere module of the instrument. All the spectra were computed at 8 cm^{-1} resolution across the spectral range 12,000–3800 cm^{-1} and ran in triplicate. Instrument control and initial spectral manipulation were performed with Result Integration software (Thermo Fisher Scientific, Dublin, Ireland). The spectra were recorded at ambient temperature and a total of 64 scans were acquired for each spectrum. For the proximate analysis comparison, off the shelf INGOT calibrations were used (Aunir, Towcester, UK) and were corrected for bias using reference materials supplied by Aunir (Haughey, Galvin-King, Malechaux, & Elliott, 2015).

2.10 Data analysis and Results (QUB)

Raw data generated by the instrument were processed using Mass Hunter suite (Agilent Technologies, Santa Clara, CA, USA) including Qual 10.0 and Personal Compound Database and Library Manager 8.1 (PCDL). For this analysis, a theoretical database including mycotoxins, plant alkaloids and other common contaminants was generated based on theoretical mass and known fragment from the literature (Figure 2). Identification was performed by comparison of the ion profile of the fragmentation for the substances experimental detected with the profile included in the database.

Figure 2: Match based on mass accuracy for atropine parent ion and sodium adduct (left).

Complementary match based on fragment (right)

2.11 Chemometrics analysis at QUB

Chemometric analysis was carried out using the software SIMCA 15 (Umetrics, Sweden). Prior to model development pre-processing was conducted. The spectral region, 2800-1800 cm^{-1} , in the FT-IR spectra and the region, 12000-9000 cm^{-1} , in the NIR spectra contain no relevant sample information and were therefore removed before data analysis. The spectral data was then processed using common scaling and pre-processing methods, and combinations thereof. The pre-processing methods used were derivatives, Savitzky-Golay (SG) and Standard normal variate (SNV). Principal Component Analysis (PCA), an unsupervised technique was used to explore differences between samples based purely on spectral differences (Black, Haughey, Chevallier, Galvin-King, & Elliott, 2016).

3 Results and discussion

3.1 Review of the analytical work conducted on the Super Cereals for the 1st incident.

This section focuses on the analytical work performed on Super Cereals involved in the incident by Queen's University Belfast (QUB), Merieux Nutrisciences and Food and Drug administration (FDA) on behalf of - CDC, and a conclusion on the root causes of the intoxications.

Mass Spectrometry analysis

The three laboratories involved in mass spectrometry (MS) measurement carried out the analysis in an independent manner and results were then compared.

Heavy metal analysis was carried out by the FDA on their sample set using ICP-MS for level of Chromium, Arsenic, Cadmium, Mercury and Lead. All results were within their maximum permitted levels thus removing the likely cause of contamination due to heavy metals (Croley et al., 2020).

In order to investigate possible small molecule polar organic contamination, untargeted screening methodologies using hyphenated mass spectrometry in conjunction with database interrogation becoming more available and easier to deploy. The overall approach followed by all laboratories to analyse these cereal samples was quite similar. Following an untargeted sample preparation, extracts were injected into GC or LC-MS using full scan mode in conjunction with a ballistic chromatographic gradient. Raw data were then processed and screened against commercial or in-house contaminant/toxin databases in order to identify possible suspect compounds and deliver a qualitative result (Christen et al., 2007).

All laboratories used LC-HRMS (LC-Orbitrap for FDA and Merieux, LC-QToF for QUB) and were able to identify the suspect analytes atropine and scopolamine with matching exact mass (less than 1ppm difference), isotopic pattern and fragmentation patterns. Identifications provided by such means of untargeted screening can only be confirmed after target analyses with certified reference materials. Pure analytical standards of suspect analytes were available at the FDA and Merieux and were used to confirm the identities of the toxins involved and further quantitation using a targeted methodology. Furthermore, Merieux and FDA laboratories carried out analysis with GC-MS and were able to confirm the identity of contaminants present in some of the cereal samples tested (Croley et al., 2020).

Quantification for the sample set obtained by FDA was carried out using LC-MS/MS analysis for which a limit of quantification for atropine and scopolamine were 3.0 µg/kg and 1.5 µg/kg, respectively. From this unique sample set, 3 samples collected by the Ugandan Health Ministry from a local district contained high level of atropine and scopolamine while retention samples as well as some local samples only contained trace or low level of toxins (Table 2).

In the absence of tropane alkaloid (TA) standards, QUB was only able to provide a qualitative result (Table 2) based on a theoretical database. Quantitative analysis provided by Merieux on the common samples

analysed by the laboratories confirmed high level of tropane alkaloids for 4 samples (WFP-0002, 0004, 0009, 0012) identified by QUB as TA contaminated. All remaining samples were also shown to be contaminated with atropine and scopolamine at relatively low-level ranging from 2.1 µg/kg to 152 µg/kg and 1 µg/kg to 17.9 µg/kg, respectively and these concentrations were not detected using LC-HRMS methodology employed by QUB. Indeed, despite recent progress in high resolution instruments, specific instrumentation for targeted analysis such as triple quadrupole or trap mass spectrometer are still considered 10 to 100 time more sensitive and are recognized as “gold standard” for quantitative analysis (Jakabova, Vincze, Farkas, Kilar, Boros, & Felinger, 2012). However, the analysis undertaken at QUB was able to detect the harmful levels (Perharič, Juvan, & Stanovnik, 2013) of contamination of the TAs.

Table 2: Mass spectrometry results obtained using qualitative methodology at QUB and quantitative methodology at Merieux (results in parentheses). FDA data is from reference (Croley et al., 2020)

<u>QUB Sample Code</u>	<u>Atropine</u>	<u>Scopolamine</u>
WFP-0001	Not detected (2.1 µg/kg)	Not detected (1 µg/kg)
WFP-0002	Detected (2100 µg/kg)	Detected (300 µg/kg)
WFP-0003	Not detected (20.8 µg/kg)	Not detected (3 µg/kg)
WFP-0004	Detected (1800 µg/kg)	Detected (250 µg/kg)
WFP-0005	Not detected (83 µg/kg)	Not detected (10.6 µg/kg)
WFP-0006	Not detected (87 µg/kg)	Not detected (10.3 µg/kg)
WFP-0007	Not detected (152 µg/kg)	Not detected (17.9 µg/kg)
WFP-0008	Not detected (80 µg/kg)	Not detected (11.6 µg/kg)
WFP-0009	Detected (8500 µg/kg)	Detected (1000 µg/kg)
WFP-0010	Not detected (99 µg/kg)	Not detected (12.1 µg/kg)
WFP-0011	Not detected (18 µg/kg)	Not detected (3.6 µg/kg)
WFP-0012	Detected (17200 µg/kg)	Detected (2400 µg/kg)
WFP-0013	Not detected	Not detected
WFP-0014	Not detected	Not detected
WFP-0015	Not detected	Not detected
WFP-0016	Not detected	Not detected
WFP-0017	Not detected	Not detected
WFP-0018	Not detected	Not detected

WFP-0019	Not detected	Not detected
WFP-0020	Not detected	Not detected
WFP-0021	Not detected	Not detected
WFP-0022	Not detected	Not detected
WFP-0023	Not detected	Not detected
FDA-001	883 µg/kg	112 µg/kg
FDA-002	28633 µg/kg	3621 µg/kg
FDA-003	54 574 µg/kg	7457 µg/kg
FDA-004	Not detected	Not detected
FDA-005	26818 µg/kg	2803 µg/kg
FDA-006	41 µg/kg	17 µg/kg
FDA-007	20 µg/kg	Trace
FDA-008	Trace	Trace
FDA-009	44 µg/kg	Trace
FDA-010	12 µg/kg	Trace

3.2 Review of the analytical work conducted on the Super Cereals from the third incident.

The third incident occurred in Uganda during August 2019 and initial reports indicated that people were presenting with symptoms very similar to those in the previous incidences again implicating contaminated SC as the cause of the illness. This raised the question of the supply chains and whether contaminated material was available from another source, as the supplier implicated was a Belgian company. Further investigation was instigated by WFP and a series of samples were shipped to QUB for analysis. These were assigned sample id WFP-0024 to WFP-0039 (see Table 3) and included retention samples (WFP-0024 to WFP -0031), suspect samples and store samples (WFP-0036 to WFP-WFP-0038).

Initial analysis of this sample set was carried out using similar screening methodology by LC-HRMS as previously used for the 1st incident. Known contaminated samples from the 1st incident (WFP-0002, 0004, 0009, 0012) were used as positive controls for atropine and scopolamine in the absence of pure reference standard. Samples WFP-0032, 0033, 0034 were found contaminated with high level of tropine alkaloid comparable to the previous incident.

Table 3: Samples sent to QUB from the third incident in August 2019 with tropane alkaloid detected by LC-HRMS

<u>QUB Sample Code</u>	<u>Original Sample Code</u>	<u>From</u>	<u>Atropine</u>	<u>Scopolamine</u>
WFP-0024	47401	Zulte, Belgium	Not detected	Not detected
WFP-0025	47493	Zulte, Belgium	Not detected	Not detected
WFP-0026	47268	Zulte, Belgium	Not detected	Not detected
WFP-0027	47982	Zulte, Belgium	Not detected	Not detected
WFP-0028	47607	Zulte, Belgium	Not detected	Not detected
WFP-0029	47564	Zulte, Belgium	Not detected	Not detected
WFP-0030	47955	Zulte, Belgium	Not detected	Not detected
WFP-0031	47107	Zulte, Belgium	Not detected	Not detected
WFP-0032	Sample 1	SC returned from beneficiary Saburuni Jackline	Detected	Detected
WFP-0033	Sample 2	SC returned from beneficiary Akwero Suzan	Detected	Detected
WFP-0034	Sample 3	SC returned from beneficiary Aber Sunday	Detected	Detected
WFP-0035	Sample 4	SC returned from beneficiary Adyee Regina	Not detected	Not detected
WFP-0036	Sample 7	SC	Not detected	Not detected
WFP-0037	Sample 8	SC	Not detected	Not detected
WFP-0038	Sample 9	SC	Not detected	Not detected
WFP-0039	Sample 11	SC returned from beneficiary Galla Mary	Not detected	Not detected

Following the analysis of the samples by mass spectrometry, further investigations using FTIR, NIRS and chemometrics were instigated to determine if samples from the third incident were related in any way to the previous incidents. By using non-targeted analyses in this way, which affords a detailed profile of the representative sample, it is possible to compare the profile of the sample in question to profiles gathered previously that represent historic material that has been shown to be fit for purpose. Appropriate chemometrics can then be used to discern if there are differences between the sample in question and historic samples.

PCA chemometric analysis was carried out on SC samples sent by WFP from the 1st and 3rd incidents. Figure 3 shows the PCA plots for the NIR and FTIR data for control/retained samples from the two suppliers, Turkey (1st incident) and Belgium (3rd incident). The resulting plots indicate that the PCA models show

distinct differences between samples from the two suppliers (Turkish and Belgian), as separate clusters were observed. These differences are likely to be due to variations in the raw materials and processing and show that the samples from the two supply chains can be distinguished from each other.

Figure 3: PCA plots of (A) NIR and (B) FTIR data from control/retained samples from the two companies (Turkey and Belgium) implicated in the incidents.

Figure 4: PCA plots for (A) NIR and (B) FTIR data for retained samples (Belgian supplier) and the 3rd incident suspect samples.

The next investigation was to observe if there were any differences between the retained samples from the Belgian supplier and the suspect samples recovered from the 3rd incident. Figure 4 shows the PCA plots

for the NIR and FTIR data for retained samples (WFP-0024 to WFP-0031, Belgium) and the 3rd incident samples identified as being suspect (WFP-0032 to WFP-0039). It was noticeable in both plots that the samples (WFP-0032 to WFP-0034), circled blue, were obtained from households where sickness had been reported and appear as distinct clusters, separated from the main Belgian SC samples. The SC store samples (WFP-0036 to WFP-0038) were found to be separated from the suspect samples (WFP-0032, WFP-0033, WFP-0034 & WFP-0039) and closer to the retained samples from the Belgian supplier i.e. distinct clusters were observed in the PCA plots, indicating that the suspect samples were different in profile to those obtained from the Belgian supplier. In the FTIR PCA plot, the sample circled green (WFP-0035) was found to be remote from the main Belgium control/retained samples and was from a household where there was no sickness reported. The sample falling outside the ellipse in the NIR PCA plot and the sample circled red in the FTIR plot was the same sample (WFP-0039) which had been found to have caused intoxication in the household it had been retrieved from. This sample appears to have a very different profile to the other samples analysed which may be explained by the observations which are made in the proximate analysis section.

Figure 5 PCA plots for (A) NIR and (B) FTIR data for the suspect samples taken in 1st and 3rd incidents.

Therefore, based on the data in Figure 4, the suspect samples and control samples from the 3rd incident appeared to be different. It was decided to make a further comparison between the suspect samples taken in the 1st and 3rd incidents. Figure 5 shows the NIR and FTIR PCA plots for the suspect samples taken in 1st incident (WFP-0001 to WFP-0012) and those taken in the 3rd incident (WFP-0032 to WFP-0039). The

samples with the blue circle in both plots were found to be from households with reported sickness in both incidents with the yellow dots represented samples (WFP-0032 to WFP-0034) from the 3rd incident obtained from households with sickness whilst the red dots represented the “mid-wife” sample (WFP-0012) (1st incident) which was the known to have caused illness, whilst the other red dot represented a suspect sample (WFP-0004, 1st incident) which had been obtained and consequently found to have a high levels of atropine and scopolamine (see Table 2). As these suspect samples clustered closely together from both incidents and are indistinguishable based on both FTIR and NIR spectral data, it strongly indicated a cross-over between different batches/bags implicated in the 1st and 3rd incidents.

In summary, the PCA plots of the data indicated a link between the incidents for which suspect samples were taken in the 1st and 3rd incidents and also that the SC samples from the two different suppliers could be distinguished using NIR and FTIR and chemometrics. Samples (WFP-0032 to WFP-0034) from the batch which had reported intoxication in the 3rd incident continuously demonstrated strong similarities with the two suspect samples from 1st incident (one labelled “mid-wife” (WFP-0012, from a household with sickness) and WFP-0004 which was found to have significant levels of atropine and scopolamine). Sample WFP-0039, obtained from a household during the 3rd incident is continually falling near or outside the ellipse of the PCA plots, indicating significant disparity between it and all the other samples which indicated that this particular sample was different to the others. Samples WFP-0032, WFP-0033, WFP-0034 and WFP-0039, which came from households with reported intoxication (3rd incident) were shown to be different from the retained samples (Belgian samples) and samples obtained from SC stores (Samples WFP-0036 to WFP-0038), which indicated that these samples did not come from the same producer.

Based on the metadata (Table 3) associated with the 3rd incident, the samples obtained from SC stores have the same producer (Belgian) as the retained samples, whereas those obtained from households with suspect samples (WFP-0032,WFP-0033,WFP-0034 and WFP-0039) had unidentified producers based on the supplied meta-data.

Proximate Analysis Results

The SC foodstuff has maximum and minimum specifications for certain analytical parameters including moisture (<10%) and protein (>14%) (Table 4). A range of Ingot Calibrations from AUNIR (UK) which are based on NIRS data and were developed to give proximate analysis of animal feed and ingredients were used as calibrants. It was decided to use one of these calibrations (Group 60: Low Protein Low Oil) to give a baseline reading on parameters associated with the SC foodstuff. For moisture analysis (Figure 6 (A)) the Belgian retained samples and store samples (WFP-0036 to WFP-0038) as well as the Turkish retained samples (1st incident, WFP-0013 to WFP-0023) have moisture levels at <10% which is within specification. It is noteworthy to point out that suspect samples (3rd incident, WFP-0032 to WFP-0034 & WFP-0039) and the “midwife” sample (1st incident, WFP-0012) have moisture levels >10% which were outside of the specification parameter. The fact that suspect samples appear to contain more moisture and coupled with the fact it was found that they contained Datura seeds (PCR test, results not shown), might be an explanation why these samples had a darker appearance. Acid Detergent Fiber (ADF) was another parameter applied to show potential differences between retained samples and suspect samples from both incidents. ADF is a measure of the percentage of the plant material that is difficult to digest, and this includes cellulose, lignin, and silica. The retained samples from both incidents (Belgium and Turkey) have lower levels of ADF (Figure 6 (B)) than the suspect samples which may be due to the latter having Datura seeds present. These suspect samples had been tested using Next Generation Sequencing (NGS) Metabarcoding Analysis carried out at Merieux indicating the presence of Datura species (results not shown). Datura species contain high concentrations of scopolamine and atropine, among other tropane alkaloids, in both the plant body and seeds, with the highest concentrations found within the seeds. With regards to the protein levels (Figure 6 (C)), the Belgian Samples (3rd incident) have indicative levels of protein that were within specification i.e. >14%. However, all Turkish retained samples and all suspect samples had protein levels below the specification level (<14%). Sample WFP-0039 was found to have parameters that were out of specification with moisture levels at ~11%, protein at ~13.5% and ADF at ~4.7% which may explain why this particular sample appeared as an outlier in the previous PCA chemometric model analysis (Figure 4).

Table 4: Specifications for SC from WFP including moisture, protein, fat, fibre and ash.

Tests	Requirements	Reference Method
Moisture	Max. 10%	ISO 712
Protein	Min. 14.0g/100g flour (Nx6.25)	AOAC 981.10
Fat	Min. 6.0g/100g flour	AOAC 954.02
Crude Fibre	Max. 4.0g/100g flour	AOAC 962.09
Total ash	4.1g/100g	ISO 2171:2007

Figure 6 Proximate analysis content (A) Moisture; (B) ADF; (C) Protein

4 Conclusions

Food poisoning events linked with food aid are extremely rare and the rigor of the investigations undertaken to identify the cause showed the seriousness with which WFP viewed the incidents. While SC was suspected early in the investigations, this had to be confirmed and the root causes of the illnesses identified. Independent analysis by 3 laboratories using high resolution mass spectrometry indicated the cause was tropane alkaloids (TA) contaminated SC. The third incident which occurred a few months later, implicating a different supply chain, was extremely worrying to the WFP. However, the investigation of this by QUB using a range of spectroscopic tools was of huge importance in that the linkage between all three incidents and the initial Turkish supply chain was established. According to WFP, it appears highly likely that some of the contaminated material that was withdrawn from distribution was stolen from a warehouse

and transported to another region of the country. Further to these investigations and to compliment the data collected, the WHO launched a call in late 2019 for further information and scientific advice on the occurrence of tropane alkaloids, risk assessments and evaluations related to SC, supplements, grains and pulses (Lipp & Petersen, 2019). This advice will help the WFP set operational limits related to its food specifications.

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Author Contributions

C.T. Elliott managed the project, collected and analysed metadata and drafted the manuscript. S.A. Haughey analysed spectroscopy data and drafted the manuscript. C. McVey acquired spectroscopy data. O.P. Chevallier acquired and analysed mass spectrometry data and drafted the manuscript.

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