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Food poisoning outbreak caused by *Aeromonas* bacteria at a funeral in Buyengo Town Council, Jinja District, Uganda, February 2024

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Abstract

Background *Aeromonas* bacteria are emerging human enteropathogens that cause food poisoning, with an incubation period of 12 h-7 days, typically 24–48 h. On February 15, 2024, Ministry of Health was notified of a suspected food poisoning incident in Buyengo Town Council, Jinja District, where 72 people developed gastrointestinal symptoms after a funeral. We investigated to identify the cause, magnitude and risk factors for the outbreak, to inform control and prevention measures.

Methods We defined a suspected case as onset of abdominal pain and ≥ 1 of the following symptoms: diarrhea, vomiting or nausea in any person who attended the funeral of a religious leader in Buyengo TC in Jinja District during Feb 11–22, 2024. We identified cases through health facility records and community searches. We collected data using interviewer-administered questionnaires. We conducted descriptive epidemiology and environmental assessments to generate hypotheses. We conducted an unmatched case-control study among funeral attendees, and microbiology and toxicology laboratory tests on 20 case-patients and 14 environmental samples.

Results We identified 65 case-patients; 5% died. Common symptoms included abdominal pain (100%), diarrhea (94%), vomiting (51%) and fever (34%). All (100%) case-patients ate at least one meal at the funeral. The epidemic curve revealed multiple peaks corresponding to the different serving times at supper and breakfast. Most cases presented within 12–86 h from Monday supper time; median incubation period was 34 h (range = 12–211 h). For both meals, beef soup served was topped-up with unboiled water and inadequately re-cooked. 62% of the cases compared to 38% of the controls ate beef stew at supper (OR = 2.7; 95%CI = 1.2–6.2). Additionally, 97% of the cases compared to 40% of the controls ate leftover beef stew for Tuesday breakfast (OR = 57; 95%CI = 5.4–600). The main source of water used at the funeral was 'Kabakubya' stream. *Aeromonas hydrophilia* and *Aeromonas caviae* were isolated in the gastric aspirate from one of the case-patients, and water from the stream.

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Conclusion This was a point source food poisoning outbreak caused by *Aeromonas species* at a funeral. The *Aeromonas* was traced to the nearby stream. Stopping use of water from the stream and enhanced water, sanitation and hygiene interventions helped control the outbreak.

Keywords Food poisoning, Outbreak, *Aeromonas*, Uganda

Background

Food poisoning, a common public health problem that can cause severe illness and death, occurs when two or more people get a similar illness after consuming the same contaminated food or drink [1]. It's estimated that food poisoning affects 600 million people, killing 420,000 every year globally [2]. Food poisoning can be caused by various agents including biological, viral, natural toxins, and chemicals that can contaminate food during production, processing, distribution, or preparation [1, 3].

In Uganda, the most common food poisoning causes are bacteria such as *Salmonella*, *Escherichia coli (E. coli)*, and *Staphylococcus aureus (S. aureus)*, *Shigella*, *Campylobacter spp*, *Bacillus cereus (B. cereus)*, and *Clostridium species (spp)* [4–6]. The bacteria exist in their natural environment such as water and can contaminate meat, eggs, dairy products, food and vegetables and cause large food poisoning outbreaks during social functions [4, 5]. Bacterial food poisoning can be infectious, toxic or mixed in nature. The toxins can be produced in the food before ingestion (pre-formed toxins) or in the gut after ingestion (enterotoxins). Toxin-producing bacteria such *as E. coli*, *S. aureus*, *B. cereus*, *Clostridium*, and *Aeromonas* usually cause acute symptoms in a few hours (2–24 h) and can mimic chemical and natural toxins [3].

Aeromonas, gram-negative toxin-producing aquatic bacteria, are emerging human pathogens known to cause food poisoning including acute gastroenteritis and septicemia. Four species including *Aeromonas caviae*, *Aeromonas dhakensis*, *Aeromonas veronii*, and *Aeromonas hydrophilia* account for > 96% of the incidents [8, 10].

Food poisoning due to *Aeromonas* usually presents with abdominal pain or cramps, diarrhea which maybe bloody, vomiting, and nausea with or without fever; incubation period of 12 h to 7 days, typically 24–48 h [7–10]. Some of the known risk factors for food poisoning include poor hygiene practices, inadequate cooking methods, improper food storage conditions, and lack of awareness among consumers [6, 8, 10].

Aeromonas spp are common in aquatic environments, yet only a few studies on food poisoning outbreaks caused by *Aeromonas spp* have been published, mostly from settings outside Sub-Saharan Africa [11–15]. With the Sub-Saharan region's growing elderly population, which is particularly vulnerable to *Aeromonas* infections, understanding the outbreak risk factors is essential for guiding effective public health interventions in these settings.

On February 15, 2024, Ministry of Health was notified of a suspected food poisoning outbreak in the Buyengo Town Council (TC) in Jinja District. The alert followed several people from the town council, complaining of acute onset of abdominal pain and diarrhea. Many of the sick reported attending a funeral in Bukasami village, Buyengo TC in the preceding days. Overall, it was reported that seventy-two people had been admitted with severe gastrointestinal symptoms, three of whom had died while new cases were still being registered. We investigated to determine the cause, magnitude and risk factors for the outbreak to inform control and prevention measures.

Methods

Outbreak area

The outbreak occurred in Buyengo TC in Jinja District and the neighbouring Nawampiti SC in Luuka District respectively. The suspected funeral took place in Bukasami village, in Buyengo TC which borders Nawampiti SC to the north (Fig. 1). The deceased was the head of household and a religious leader, and his funeral lasted several days attracting a lot of attendees, mainly from Buyengo TC and Nawampiti SC. He was buried on Monday, February 12, 2024, one day after his death but a prayer ceremony was held on the following day. On both occasions, funeral attendees were served different foods and drinks at breakfast, lunch, and supper.

Case definition and finding

We defined a suspected case as onset of abdominal pain and ≥ 1 of the following symptoms: diarrhea, vomiting or nausea in any person who attended the funeral of a religious leader in Buyengo Town Council (TC) in Jinja District during Feb 11–22, 2024. A confirmed case was a suspected case with laboratory confirmation of *Aeromonas species* in a clinical specimen.

We conducted case finding using record reviews at the three health facilities serving the affected area including one health center III, one general hospital, and a regional referral hospital, and community case search. At the health facilities, we reviewed patient registers and files to identify case-patients using the case definition. In the community, we worked with the village health teams and conducted house-to-house active case search in the villages. We also used snowballing to identify additional cases especially among visiting mourners.

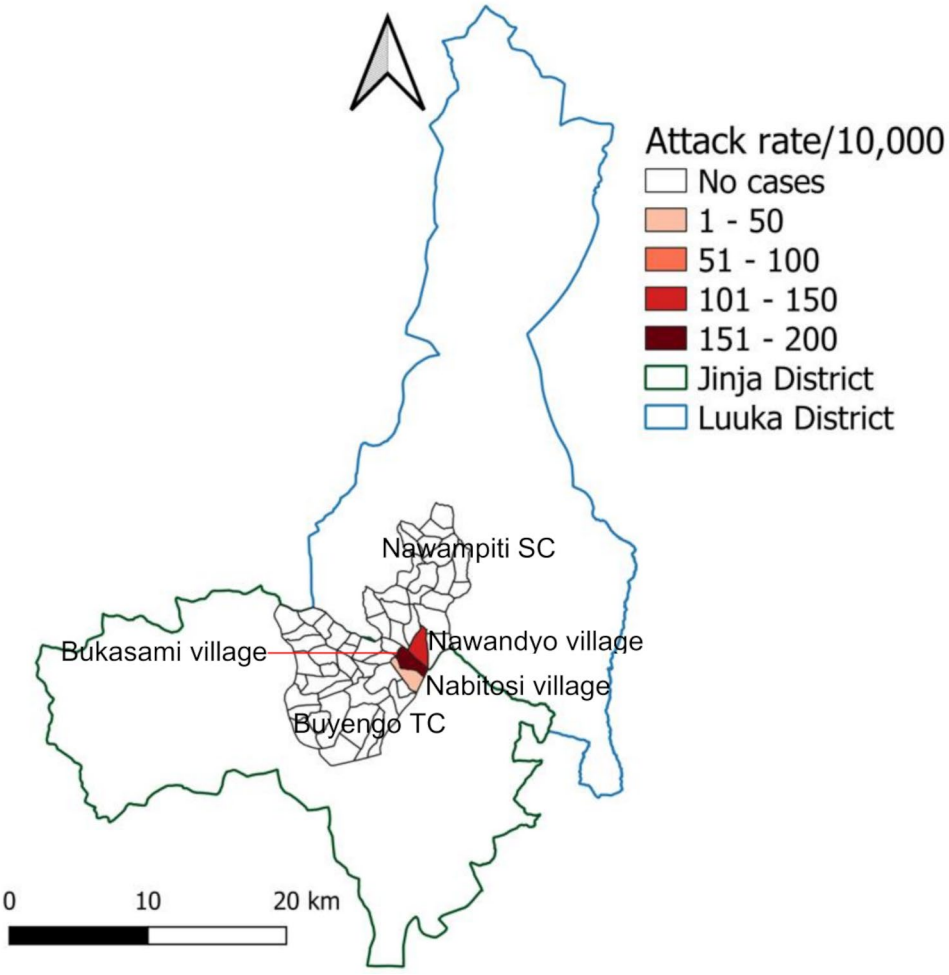

Fig. 1 Attack rate by Village in Buyengo Town Council and Nawampiti Sub-county during an outbreak of poisoning caused by *Aeromonas* bacteria, Jinja and Luuka districts, Uganda, February 2024

The identified case-patients were physically interviewed using a case investigation form and a line list developed. The case investigation form included information on demographic characteristics, clinical features such as the time of onset and duration, social history, foods and drinks taken on specific days and their source, any laboratory investigations done, treatment given, and outcomes.

Descriptive epidemiology

We conducted descriptive analysis of data from interviews with case-patients. We characterized cases by person, place, and time and possible exposures. Person characteristics included sex and age. We categorized age (in years) into six meaningful categories including 0–4 (younger children), 5–14 (older children), 15–24 (young persons), 25–39 (young adults), 40–59 (middle aged) and ≥60 (elderly). We used the 2023 population estimates from the Uganda Bureau of Statistics (UBOS) for Jinja and Luuka districts to calculate attack rates by sex, age group, subcounty and village [16]. We constructed choropleth maps using the Quantum Geographic Information System (QGIS) software to display attack rates by place. We used an epidemic curve to analyse the distribution of cases by time of symptom onset.

Estimation of the incubation period

We collected data on the date and serving time for the various foods taken at the funeral for all the case-patients using a questionnaire. We also collected data on the date and time of onset of the first symptom among the case-patients. We based on the hypothesis generation findings to identify the food(s) that were the likely source of exposure. The incubation periods were estimated as the time interval (hours) between the serving time for suspected food and onset of the first symptom for each individual. For case-patients who took more than one serving of the suspected food at the funeral, we assumed that they were exposed at the earliest serving. We conducted sensitivity analysis by comparing the overall estimates of the

incubation period and those of case-patients who took only one serving of suspected food to ensure consistency. We arranged the individual incubation periods in ascending order to ascertain the median and range using Microsoft Excel.

Case management

A treatment unit was established at Buwenge General Hospital, the main hospital in Jinja District to manage the case-patients. We reviewed hospital records and interviewed attending clinicians about the care and treatment interventions.

The respondents included the medical superintendent of the hospital, three attending doctors and a nurse. We obtained information on how the case-patients were transported to the hospital, time interval from symptom onset to admission, treatment given, and availability of the necessary drugs and health supplies and clinical outcomes.

Environmental assessment

We observed the funeral site and interviewed several key informants including two widows of the late religious leader and one of the cooks. We also interviewed the local council chairpersons of Bukasami and Nawandyo villages and household heads/caretakers of the deceased case-patients. Interviews focused on circumstances surrounding the death of the religious leader, burial practices, source of water, foods prepared at the funeral, the food preparation and handling processes. We also searched for any dumped leftover food at the funeral site. We collected water samples from the different sources of water used at the funeral, including four boreholes and 'Kabakubya" stream in Bukasami village.

Laboratory investigations

We collected both clinical and non-clinical samples from case-patients and the environment for laboratory testing. Since the etiology was unclear and clinical syndrome nonspecific, we collected all the four types of specimens recommended for investigating food poisoning outbreaks. These included gastric aspirate from one case-patient and stool samples from eight case-patients. Additionally, we took off samples for blood culture from 2 case-patients, blood chemistry from 20 case-patients, complete blood count from 5 case-patients, toxicology from 10 case-patients and urinalysis from four case-patients. The non-clinical samples included four leftover food samples, piece of a soiled mattress used by one of the case-patients, and ten water samples. The water was collected from five water sources said to have been used during the funeral, and one jerrycan from the household where the funeral was held. The clinical and non-clinical samples including water samples were sent to Uganda's Central Public Health Laboratories (CPHL) for microbiology. Additionally, some clinical samples and the non-clinical samples such as leftover food were sent to the Directorate of Government Analytical Laboratory (DGAL) for toxicology screening.

Sample collection, packaging, transportation and storage

Gastric aspirate

The gastric aspirate sample was collected by the attending medical officer after obtaining consent from the patient's caregiver through a nasogastric tube. The procedure was conducted by instilling approximately 250 mL of saline with immediate lavage of that same quantity of fluid. Twenty milliliters of the first fluid lavage was collected in a sterile sample container. The technique was repeated until the recovered solution was clear of particulate matter or pill fragments. The sample was kept and transported at 2–8 ° C to the reference testing laboratory within 6 h of sample collection.

Stool

Stool samples were collected into 30 ml capacity clean plastic stool containers with screw caps. The patients were instructed on how to properly collect and transfer an adequate stool sample into the container using a small spoon that comes with the containers. The stool was first collected on a piece of toilet paper and about 5 g transferred into the stool container using the provided spoon, and immediately capped. The samples were kept at 2–8 ° C and transported in cold boxes with conditioned ice packs to the reference testing laboratory within 6 h of sample collection.

Urine

Urine samples were collected into 50 ml capacity sterile urine containers with screw caps. The patients were instructed on how to properly collect clean-catch, midstream urine and immediately cap the container without touching inside the container. The samples were kept at 2–8 ° C and transported in cold boxes with conditioned ice packs to the toxicology reference testing laboratory within 6 h of sample collection.

Blood

The blood samples were collected by laboratory staff at Jinja Regional Referral Hospital under aseptic technique from the antecubital vein. A tourniquet was applied 10 cm above the elbow and the cubital fossa and adjacent area (up to 5 cm) was disinfected with chlorhexidine solution using a piece of cotton wool for at least one minute and let to dry, and this was repeated three times. For blood culture, 8 ml of venous blood were drawn into a sterile blood culture bottle (BD BACTEC AEROBIC/F) for adults while 3 ml were drawn into the blood culture

bottle (BD BACTEC PEDS PLUS/F) for children. The blood culture samples were immediately placed into the incubator (BD BACTEC FX 200) at 37∘C. Samples were declared negative only after 5 days of no growth.

For chemistry and toxicology, 4 ml of venous blood were drawn into plain (red-top) vacuutainers. The chemistry samples were immediately placed into the chemistry machine (KOBAS C311) and analyzed. Toxicology samples were kept at 2–8 ° C and transported in cold boxes with conditioned ice packs to the reference testing laboratory within 6 h of sample collection. For complete blood count (CBC), 4 ml of venous blood were drawn into ethylenediamine tetra-acetic acid (EDTA) tubes (purple-top) and immediately placed into the CBC machine (SYSMEX XN 550) for analysis.

Water samples

Following standard safety procedures, with new gloves before opening each of the sterile water sample bottles, sampling sites were selected. If wading was noted, we waited until the disturbance in the bottom sediments was minimal and the water relatively undisturbed. To collect water from the stream and other surface water collection points, the water sample containers were inverted and in a one quick motion, the mouth was plunged into the water about six inches below the surface. The containers were then tipped to fill and removed out of the water. The excess sample was poured off to near the shoulder level and immediately capped. For Boreholes, the water sample containers were directly filled with water pumped from the boreholes. The excess sample was poured off to near the shoulder level and immediately capped. The samples were packed in cold boxes with conditioned ice packs and shipped for analysis at the reference laboratory within six hours of sample collection.

Microbiology testing

Water samples were filtered using the membrane filtration method and cultured to isolate and quantify bacteria. The filtered water samples, and clinical samples were cultured on XLD agar for *Salmonella and Shigella*, Chromogenic *E. coli* agar for *E. coli*, and ESBL selective agar for extended-spectrum-beta-lactamase producing bacteria. Furthermore, MALDI-TOF mass spectrometry and Phoenix M50 methods were also used to identify additional bacteria.

Toxicology screening

Toxicology screening was conducted using Gas Chromatography-Mass Spectrometry Mass Spectrometry (GCMS/MS) method.

Hypothesis generation

We conducted 61 interviews among case-patients that were identified through active case-search, including all the three deceased case-patients. We also interviewed members of the district health team that were among the first responders, clinicians that attended to the case-patients, and the medical superintendent of Buwenge General Hospital where most of the case-patients were managed. We obtained data on social functions attended, the circumstances surrounding the death of the religious leader, funeral activities, foods and drinks taken prior to symptom onset, specific foods and drinks served at the funeral including the source, source of water used for cooking and hand washing, food preparation and handling during the funeral, clinical presentation of cases, and case management.

We used the results of the descriptive analysis of case-patient data, key informant interviews, and environmental findings to generate hypotheses.

Case control study

To test the hypotheses, we conducted an unmatched case-control study among the funeral attendees in the two most affected villages of Bukasami and Nawandyo in Buyengo TC and Nawampiti SC respectively during February 17—23, 2024, five days following the burial of the deceased and four days after the emergence of the outbreak. The case control study design was adopted because the outcome (food poisoning) was rare and the odds ratios would appropriately estimate the risk. Additionally, it was not feasible to conduct a retrospective cohort study because there was no registration of funeral attendees upon which generation of the cohort would be based, and generating such a list retrospectively was logistically and practically challenging. We assumed that 50% of the controls were exposed, and calculated the sample size at 95% confidence level and 80% power. We adopted an odds ratio of 2.402 for the association between eating contaminated cow carcass meat and *Aeromonas* food poisoning based on similar studies in Bhutan, 2016 [13]. For each case-patient, we selected 3 control persons (case to control ratio = 1:3). The overall sample size was 244 including 61 cases and 183 controls. A control was a resident or visitor to Bukasami and Nawandyo villages in Buyengo TC and Nawampiti SC, who attended the suspected funeral but had no history of abdominal pain, diarrhea or vomiting during February 11–22, 2024. We used village house-hold lists to generate sampling frames per village and systematically sampled non-case households from where we enrolled controls. The sampling interval was determined by dividing the total number of non-case households by the control sample size. From each non-case household, we selected one control person using simple random sampling by lottery

method. An interviewer administered questionnaire was administered to the eligible case-patients and control-persons to obtain information on their demographic, clinical characteristics, and foods and drinks taken at the funeral among others (Supplementary File 1). We analyzed the data using Stata version 17 to identify risk factors associated with food poisoning among the funeral attendees. We used odds ratios to determine the relationship between different exposures and food poisoning by conducting bivariate and multivariate logistic regression analysis. We chose odds ratios due to the nature of the study design but also because they would approximate the risk of food poisoning among the funeral attendees since the outcome is rare. At multivariate analysis, we included only factors that had a p-value of ≤ 0.2 at bivariate analysis based on the statistical criteria. The multivariate analysis was conducted in a stepwise approach and exposures that did not improve the fit of the regression model as determined by the log likelihood ratio test were dropped. We also paid close attention to the changes in precision of the models as new exposures were being added. We excluded foods eaten at breakfast on Tuesday from the multivariate analysis models due to collinearity since the brown rice was served pre-mixed with beef stew. We used complete case analysis to handle missing data for analysis variables among the cases and controls; specifically, cases or controls with missing data on a certain variable were excluded in the analysis for such a variable. The associations were only considered significant at a p-value of < 0.05. We also conducted common reference group analysis to untangle possible mixing of effects of different exposures on food poisoning among the funeral attendees.

Results

Descriptive epidemiology

Person and clinical characteristics of case-patients in a food poisoning outbreak caused by Aeromonas bacteria, Jinja and Luuka Districts, Uganda, February 2024 (n = 65)

Overall, 65 case-patients were identified including one confirmed case; 3 case-patients died representing a 5% case-fatality rate (Fig. 2). The confirmed case-patient

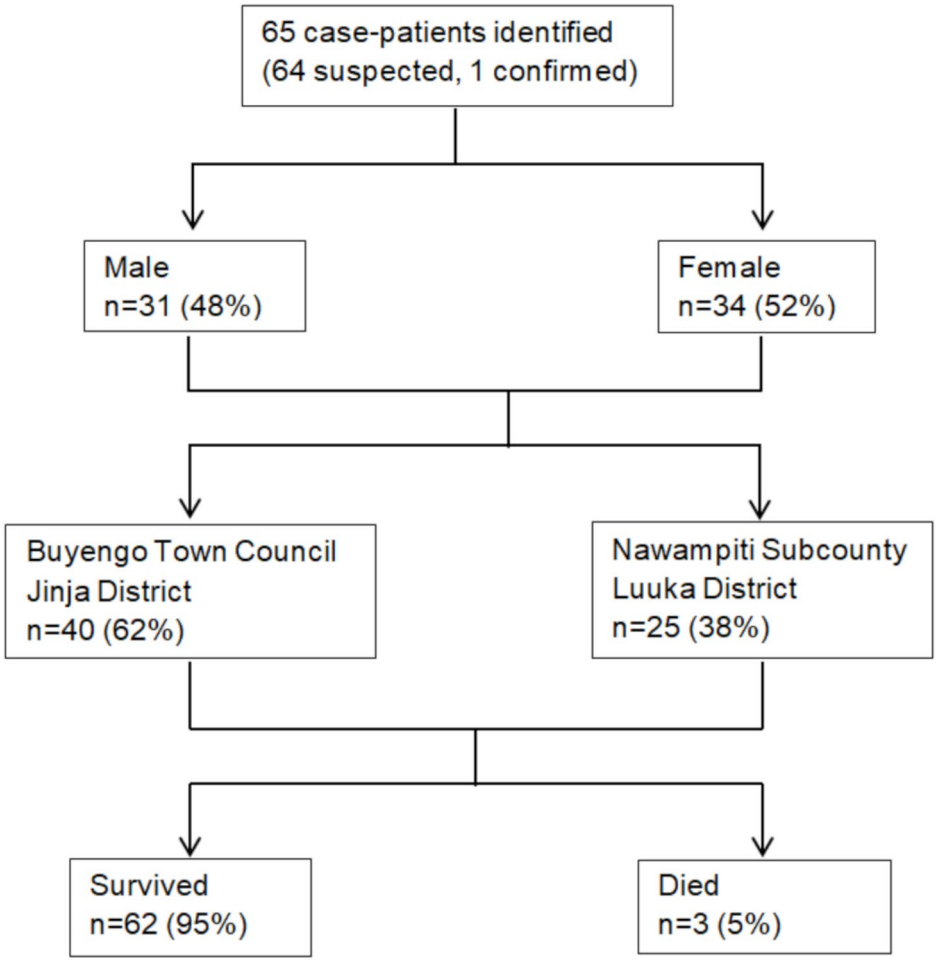

Fig. 2 Case-patients during a food poisoning outbreak caused by *Aeromonas* bacteria, Jinja and Luuka districts, Uganda, February 2024

Table 1 Attack rates by sex and age group during a food poisoning outbreak caused by *Aeromonas* bacteria, Jinja and Luuka districts, Uganda, February 2024

Variable	Population	Cases (%)	Attack rate/10,000
Sex			
Male	31,600	34(52)	11
Female	30,400	31(48)	10
Age (years)			
≥ 60	2170	6(9)	27
15–24	13,516	16(25)	12
5–14	16,988	22(34)	13
25–39	12,028	11(17)	9.1
40–59	6882	6(9)	8.7
0–4	10,416	4(6)	3.8
Median (IQR)		20 (IQR = 9–36)	

had only taken leftover fried rice pre-mixed with leftover beef stew from the supper that was served on Monday, February 12, 2024. The leftover fried was carried home by the father from the funeral on Tuesday, February 13, 2024. The majority (52%) of case-patients were female. The median age was 20 years (IQR = 9–36) and most were aged 5–14 years (Table 1).

In addition to severe abdominal pain/cramps, the majority (94%) of the case-patients reported diarrhea and vomiting (51%). Fever was reported in only 34% of the case-patients. Among the non-specific symptoms, general body weakness was the commonest (63%) followed by headache at 42% (Fig. 3). In 92% (60/65) of the case-patients, the first symptom was severe abdominal pain/cramps, with only 5% experiencing diarrhea as the first symptom. Among those with diarrhea, 25% had bloody diarrhea. Overall, females (AR = 11/10,000) and males (AR = 10/10,000) were similarly affected. The elderly (≥ 60 years) (AR = 27/10,000) were the most affected, while the 0-4-year age-group was least affected (Table 1).

Distribution of case-patients by time of symptom onset during the food poisoning outbreak caused by Aeromonas bacteria, Jinja and Luuka Districts, Uganda, February 2024

All the case-patients attended the funeral of a religious leader in Jinja District who died on Sunday February 11, 2024. Funeral attendees started arriving on the same day, but no meals were served on that day. The following day on Monday February 12, 2024, at around 10:00 h, funeral attendees were served breakfast with black tea and no accompanying food item, and lunch with foods including: brown (fried) rice, white (boiled/unfried) rice, goat's meat and beef stew from 13:00–14:00 h. Later on, the same day, supper comprising brown rice and beef stew was prepared separately and served from 20:00–21:00 h. However, some of the brown rice and beef stew that had been prepared for supper were kept and served to the funeral attendees at breakfast the following day, Tuesday February 13, 2024, between 8:00–10:00 h. For both Monday supper and Tuesday breakfast, the beef stew was topped up with water to meet the high demand and was not properly re-cooked. We found that all (100%) case-patients ate at least one meal at the funeral, and 90% (55/61) had eaten either supper on Monday or breakfast served the following day using leftover food and beef stew from the previous supper (Table 2).

Case-patients started experiencing symptoms on Tuesday February 13 2024 at 08:00 h. The epicurve revealed

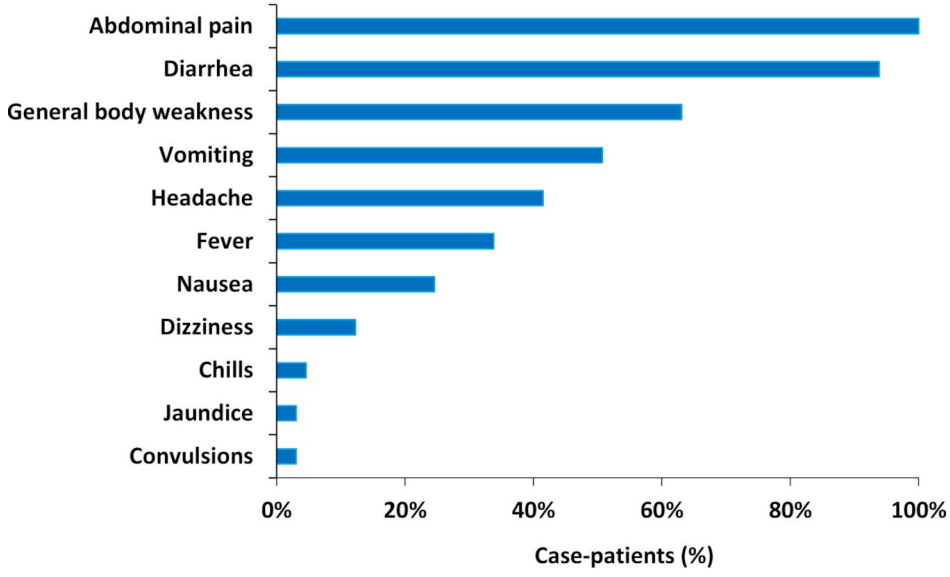

Fig. 3 Clinical presentation of case-patients during a food poisoning outbreak caused by *Aeromonas* bacteria, Jinja and Luuka districts, Uganda, February 2024

Table 2 Potential exposures associated with food poisoning caused by *Aeromonas* bacteria, Jinja and Luuka districts, Uganda, February 2024

Potential exposure	Cases	Percentage
Ate any food at the funeral (*N* = 61)	61	100
Ate either Mon supper or Tue breakfast (*N* = 61)	55	90
Ate any food onTue-13 (*N* = 61)	47	77
Ate breakfast on Tue (*N* = 47)	38	81
Ate Lunch on Tuesday (*N* = 47)	25	53
Ate Supper on Tuesday (*N* = 47)	23	49
Ate any food on Mon-12 (*N* = 61)	44	72
Ate supper on Mon (*N* = 44)	43	98
Ate Lunch on Monday (*N* = 44)	21	48
Took any drink at the funeral (*N* = 61)	26	42
Attended the funeral on Wed (*N* = 61)	14	23

multiple peaks with a time interval of about 12–24 h between; suggesting a point source outbreak with multiple exposure times corresponding to the different serving times for the case-patients at supper and breakfast. Additionally, most of these cases presented within a time interval of 12–86 h from when Monday supper was served and 2–70 h from when breakfast was served the following day on Tuesday (Fig. 4).

The estimated median incubation period for case-patients was 33 h (range = 12–185 h) from their respective serving times for Monday supper and 36 h (range = 13–211 h) from their serving times for Tuesday breakfast. We noted that seven (11%) of the case-patients developed symptoms before or around the serving time for Tuesday breakfast but had taken supper at least 12 h prior, suggesting that they had been exposed at supper time (Fig. 4). In addition, all case-patients whose estimated incubation periods were less than12 hours from their breakfast serving time on Tuesday had also taken supper the previous day. Considering Monday supper as the earliest point of potential exposure, the overall median incubation period was estimated at 34 h (range 12–211 h).

Attack rates by village and subcounty during a food poisoning outbreak caused by Aeromonas bacteria, Jinja and Luuka Districts, Uganda, February 2024

Buyengo TC in Jinja District (AR = 11/10,000) and Nawampiti Subcounty.

(AR = 10/10,000) were similarly affected. However, Bukasami Village in Buyengo TC where the funeral was held was the most affected village (159/10,000) (Fig. 1).

Environmental findings

We found that whereas the main source of water routinely used by residents was the boreholes, most of the water used at the funeral was fetched from 'Kabakubya' stream using boda bodas and a pick-up motor vehicle. This was reported to have been necessitated by urgency and increased demand for water because the funeral attracted > 1000 funeral attendees. The persons involved in food preparation at the funeral reported that unboiled water from 'Kabakubya' stream was added to top-up the beef soup at Monday supper and the beef stew was not allowed enough time to be re-cooked. The beef stew was served with freshly prepared brown rice at supper on Monday and leftover brown rice from the same supper at breakfast the following day. It was reported that all the

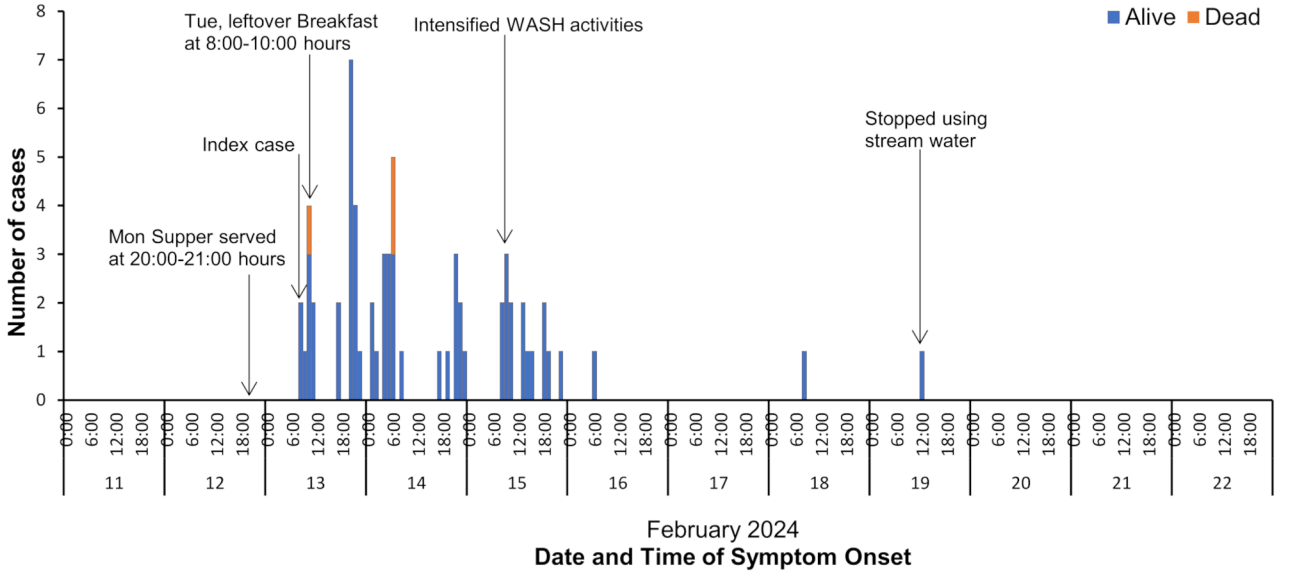

Fig. 4 Distribution of case-patients by time of symptom onset during a food poisoning outbreak caused by *Aeromonas* bacteria, Jinja and Luuka districts, Uganda, February 2024

leftover brown rice served for breakfast was already premixed with the beef stew.

Laboratory investigation findings

Aeromonas hydrophilia and Aeromonas caviae were isolated from the gastric aspirate of the deceased 4-year-old case-patient, revealing mixed infection by different strains of *Aeromonas* bacteria. Similarly, the water sample from Kabakubya water stream tested positive results for *Aeromonas hydrophilia by culture*. Overall, 3 colony forming units per milliliter (cfu/ml) of *Aeromonas* bacteria grew from the gastric aspirate while water from 'Kabakubya' stream grew 1 cfu/ml. Both the aspirate and water sample tested negative for other common bacterial causes of food poisoning including *Shigella*, *Escherichia coli*, *Salmonella spp*, *Vibrio cholerae and Campylobacter spp.* The samples from the leftover food that was found dumped at the funeral site one week after the incident tested negative on toxicology for organophosphates, carbamates, organochlorides and pyrethroids commonly found in pesticides, as well as the common herbicides. The samples were not useful for microbiology due to obvious contamination. However, the suspected food particularly beef stew was never sampled as there were no leftovers of it by the time the investigation started four days later following the incident. Blood, stool and urine samples tested negative on microbiology and toxicology testing. Failure to detect the causative agent from these samples could be explained by the fact that all were collected after case-patients had received antibiotic treatment unlike the gastric aspirate from the confirmed case. The findings from chemistry tests including renal function tests and liver function tests were in normal range.

Case management

Suspected cases were evacuated from the community to Buwenge General Hospital by the district and Red Cross health teams using ambulances. While at Buwenge General Hospital, the case-patients were admitted for observation and management. The case-patients were treated mainly with intravenous fluids to correct severe dehydration, and antibiotics including Ceftriaxone and Ciprofloxacin for suspected bacterial infection. All the case-patients were admitted for at least one day for treatment and observation.

Four (6%) of the case-patients were referred to Jinja Regional Referral Hospital for further management. The four case-patients had an average interval of 20 h from onset of profuse diarrhea to admission. They were severely ill on admission with features of shock, and required emergency resuscitation.

Three (5%) case-patients died while on admission at Buwenge General Hospital. These included 4-year old boy and 6-year old girl from the same family in Nawampiti Subcounty, Luuka District, and a 20-year old man from Buyengo TC. Of the deceased, the first one died on February 13, 2024 while the other two on February 14, 2024. The dead were found to have sought health care late (12–24 h) from symptom onset. None of the deceased case-patients was involved in the food preparation at the funeral, and their symptoms started at least a day after several other people had reported similar symptoms.

Additionally, the health facility management reported stock out of medicines and health supplies including intravenous fluids for rehydration and antibiotics during the time of the outbreak.

Hypothesis generation findings

We found that all the case-patients had taken at least one meal at the funeral on either Monday, February 12, 2024 or Tuesday, February 13, 2024. Most (90%) of the case-patients had taken either Monday supper or Tuesday breakfast served with beef stew. The beef stew had been prepared at supper on Monday, February 12, 2024 but the leftovers were served at breakfast the following day (Table 2). It was reported that during both meals, the beef stew was topped up using water from a local stream to meet the demand from the funeral attendees, and was never properly recooked.

We thus hypothesized that the food that was served at either supper on Monday, February 12 or breakfast on Tuesday, February 13, 2024 was contaminated by an infectious causative agent. The agent was likely in the unboiled stream water that was used to top-up beef stew that was not properly re-cooked, and served at supper on Monday and as leftover breakfast the following day.

Risk factors for food poisoning caused by *Aeromonas* bacteria among case-patients, Buyengo TC, Jinja District and Nawampiti SC, Luuka District, Uganda, February 2024

86% of the cases compared to 43% of the controls ate food at the funeral on Monday (cOR = 7.2; 95% CI = 3.2, 16.3). Similarly, 96% of the cases compared to 50% of the controls ate food at the funeral on Tuesday (cOR = 24; 95% CI = 4.9, 112.6). Among those who ate food on Monday, 62% of cases compared to 38% of the controls ate beef at supper (aOR = 2.7; 95% CI = 1.2, 6.2). Furthermore, 97% of the case-patients compared to 40% of the control-persons ate leftover beef stew for Tuesday breakfast (cOR = 57, 95% CI = 5.4, 600).

An equivalent proportion of case-patients (97%) compared to control-persons (40%) ate leftover brown rice pre-mixed with the leftover beef stew for Tuesday breakfast (cOR = 57, 95% CI = 5.4, 600). However, eating brown rice at supper was not significantly associated with food poisoning (aOR = 1.2, 95% CI = 0.1, 11) (Table 3). Similarly, common reference group analysis also revealed that

Table 3 Risk factors for food poisoning caused by *Aeromonas* bacteria among case-patients, Buyengo TC, Jinja district and Nawampiti SC, Luuka district, Uganda, February 2024

Exposure	Cases (%)	Controls (%)	cOR (95% CI)	aOR (95% CI)
Ate beef stew for Mon supper				
Yes	26(62)	23(38)	2.6 (1.2,5.9)	2.7(1.2,6.2)
No	16(38)	37(62)	Ref	
Ate brown rice for supper on Monday				
Yes	36(86)	44(73)	2.2 (0.8,6.2)	1.2 (0.1,11)
No	6(14)	16(27)	Ref	
Ate white rice for supper on Monday				
Yes	6(14)	16(27)	0.5 (0.2,1.3)	0.5 (0.052, 4.7)
No	36(86)	44(73)	Ref	
Ate leftover beef stew for breakfast on Tue				
Yes	38(97)	4(40)	57 (5.4,600)	
No	1(3)	6(60)	Ref	
Ate leftover brown rice for breakfast on Tue (all served with leftover beef stew)				
Yes	38(97)	4(40)	57 (5.4,600)	
No	1(3)	6(60)	Ref	
Took black tea at breakfast on Tuesday				
Yes	11(69)	13(59)	1.5 (0.39,5.9)	
No	5(31)	9(41)	Ref	

[#]cOR refers to crude Odds Ratio

*aOR refers to adjusted Odds Ratio, [*]Beef stew and brown rice pre-mixed beef stew eaten on Tuesday were not included in the multivariate models despite having a significant association with food poisoning at bivariate analysis, due to collinearity

Table 4 Common reference group analysis for risk factors of food poisoning among funeral attendees in Buyengo TC and Nawampiti SC, February 2024

Variable	Cases (%)	Controls (%)	aOR*	95% CI
Didn't eat brown rice or beef on Mon supper	2(5)	10(17)	Ref	
Ate brown rice, no beef	14(33)	27(45)	2.6	0.5, 13
Ate beef, no brown rice	4(10)	6(10)	3.3	1.1, 24
Ate both brown rice and beef	22(52)	17(28)	6.5	1.2, 34

*aOR refers to adjusted odds ratio

Table 5 Dose-response assessment for the relationship between beef and food poisoning among funeral attendees in Buyengo TC and Nawampiti SC, February 2024

Exposure	Cases (%)	Controls (%)	aOR	95%CI
Never ate beef stew	1(4)	1(17)	Ref	
Ate one meal of beef stew	9(32)	2(33)	4.5	0.19, 107
Ate two meals of same beef stew	18(64)	3(50)	6.0	0.29, 124

eating the brown rice without beef stew for Monday supper was not significantly associated with food poisoning (aOR = 2.6; 95%CI = 0.50,13) (Table 4). Dose-response assessment of the relationship between beef stew and food poisoning revealed an additive effect of consuming beef stew on the odds of food poisoning among the funeral attendees. Whereas eating one meal of beef stew led to a 4.5-fold increase in the odds of food poisoning compared to those who never ate beef stew; eating two meals of the same beef stew increased the odds 6-folds, though this was not statistically significant (Table 5).

Discussion

Our investigation revealed a point source outbreak of food poisoning caused by *Aeromonas* bacteria in Buyengo TC in Jinja District and Nawampiti SC in Luuka District. Cases presented with symptoms of acute gastroenteritis, mostly severe abdominal pain/cramps and diarrhea, some of which was bloody. The overall case-fatality rate was 5% and the elderly people were the most affected age-group. The outbreak affected three neighboring villages and followed a funeral ceremony that was held for several days at Bukasami Village in Buyengo TC. Bukasami village, where the funeral was held was the most affected village.

We found that all case-patients had eaten at least one meal at the funeral either on the day of burial, Monday February 12, 2024 or the day after. Eating beef stew at Monday supper and/or leftover beef stew at breakfast on the following day was a risk factor for food poisoning. We found that funeral attendees who ate beef stew at the funeral were more likely to develop food poisoning. This could suggest that the beef stew was contaminated with *Aeromonas* from the unboiled water that was added top up the soup without proper re-cooking. We traced the source of the *Aeromonas* to 'Kabakubya' stream from which most of the water used at the funeral was fetched.

Aeromonas are gram-negative, non-spore forming rods that live in aquatic environments worldwide [9, 10]. Nineteen of the thirty-six known species are considered emerging pathogens to humans [8]. Several studies have implicated *Aeromonas* species in causing food poisoning, wound infection and septicemia [7–10, 13]. More than 96% of the incidents were caused by one of four species including *Aeromonas caviae*, *Aeromonas dhakensis*, *Aeromonas veronii*, and *Aeromonas hydrophilia* [8, 10, 17].

In this outbreak, the case-patients mainly experienced severe abdominal pain/cramps, and diarrhea in addition to vomiting. This symptom profile is consistent with the clinical features of acute gastroenteritis caused by *Aeromonas* bacteria. The bacteria are known to cause acute gastroenteritis with features including abdominal pain, diarrhea which maybe bloody or not, vomiting, nausea, and occasionally jaundice and dyspnea. The bacteria also produce enterotoxins and can cause severe symptoms in a short time including sepsis and death [8].

The bacteria have a low infective dose following natural exposure with a median dose of only 0.9 colony forming units per milliliter (cfu/ml) required for 1% illness risk [9, 10, 12]. This infectious dose following natural exposure is thousand times lower than what has been estimated from challenge studies [18]. Studies have also found that the infectious dose for *Aeromonas* in diarrheal illnesses is comparable to that of well-known enteropathogenic bacteria such *as Campylobacter* and *Salmonella* species [8, 18]. The gastric aspirate from the confirmed case-patient in this outbreak grew 3 cfu/ml on culture, which was over three times the median infective dose.

The estimated median incubation period of 34 h and the range of 12–185 h for 98% of the cases was consistent with the known incubation period for *Aeromonas* which ranges from 12 h to 7 days [19, 20]. Furthermore, most of the cases presented within two days from the potential exposure which is consistent with findings of a previous study in Bhutan in which 70% of the cases developed symptoms within 2 days of consuming beef stew from a carcass, contaminated with *Aeromonas* [13].

Contaminated water sources are an important source of *Aeromonas* [15]. The most frequent route of entry of *Aeromonas* into humans is oral–fecal route, but the bacteria can also enter the body through wounds [9, 10, 12]. Consumption of contaminated water and food are considered the main routes of transmission [21, 22]. In this outbreak, case-patients consumed beef stew prepared using water from a stream which was subsequently found to contain *Aeromonas hydrophilia*. This was consistent with the known oral-fecal transmission of *Aeromonas* [22]. Similarly, a food borne diarrheal disease outbreak investigation in a college in China found that students were exposed from consuming cucumber salads that had been rinsed with water contaminated with *Aeromonas* [11].

In this particular outbreak, we found mixed infection with *Aeromonas hydrophilia* and *Aeromonas caviae* in the gastric aspirate from the only confirmed case-patient. This particular case-patient was also among the three that died. Studies have shown that mixed infections by different *Aeromonas* species can occur and usually result into more severe disease as compared to when the infection is caused by a single species [8, 18]. Whereas *Aeromonas* bacteria are known to infect both immunocompromised and immunocompetent people, the former are usually more affected. This explains why the attack rate was highest in the elderly.

All the case-patients were admitted for at least one day and treated with antibiotics and intravenous fluids. Whereas the majority, 62(95%) improved within 48 h, three case-patients died. We ascertained that the deceased case-patients had no known underlying medical condition, and had no symptoms or signs of illness prior to attending the funeral. These fatalities could be explained by the delayed health-care seeking since all the deceased case-patients sought health care beyond 12–24 h from symptom onset, and were severely dehydrated at admission. Additionally, the treatment centers reported stock out of medicines and health supplies at the time, with caretakers being advised to buy the prescribed drugs from private providers leading to further treatment delay. This suggests that the deceased died of severe dehydration, resulting from late care seeking and delayed treatment initiation at the health facilities. This probable cause of death is consistent with the known causes of death among patients with diarrhea which include severe dehydration, electrolyte imbalance and occasionally septicemia [23].

The last case in this outbreak presented 10 days from the last potential point of exposure, beyond the typical incubation period of *Aeromonas*. However, studies have also found evidence of bacterial shedding by symptomatic and asymptomatic persons leading secondary transmission [8].

Study limitations

Ongoing criminal investigations into the incident interfered with the investigation. Subsequently, we were unable to reach the supplier of the suspected beef and many of the cooks as they denied participating in food preparation due to fear of legal consequences. However, we were able to interview the main cook involved in preparing the implicated meals.

The investigation started four days later after the incident had occurred, and several visiting funeral attendees had already left the outbreak area and couldn't be reached for interviews. This might have underestimated the magnitude of the outbreak.

No food samples were collected from the suspected food particularly the beef stew. The only food samples sent for testing were found dumped at the funeral site one week after the incident, and weren't useful for microbiology. Nevertheless, they were still useful for toxicological investigations which were negative.

There was no registration of attendees at the funeral and we thus could not compute attack rates based on specific at-risk population denominators. Nevertheless, we calculated population level attack rates to estimate the risk of the different subgroups of persons during this outbreak. Whereas we might have underestimated the true risk, our findings are consistent with published literature on *Aeromonas* food poisoning and biologically plausible.

With the exception of the single confirmed case, clinical samples from the rest of the case-patients were collected at least four days following the outbreak and after they had been treated with antibiotics. This could explain the very low confirmation rates of the causative agent for this outbreak. Nevertheless, the isolation of *Aeromonas* bacteria from the gastric aspirate of one of the deceased case-patients coupled with negative test results for other common bacterial causes of food poisoning such as *Shigella*, *E. coli*, *Salmonella*, *and Campylobacter* in both the clinical and non-clinical samples, plus the evidence from environmental assessment strongly links *Aeromonas* bacteria to the food poisoning among the funeral attendees.

Whereas funeral activities occurred during February 11–13, 2024, data collection commenced on February 17, 2024; 5 days following the burial of the deceased and four days after the emergence of the outbreak. This could have resulted into recall bias of the foods eaten at the funeral among the cases and controls.

Conclusion

This was a point source outbreak of food poisoning in Buyengo TC and Nawampiti SC in Jinja and Luuka districts respectively caused by *Aeromonas species* at a funeral. The source of the *Aeromonas* was the unboiled water from 'Kabakubya' stream which was added to top-up the beef soup at Monday supper and Tuesday breakfast. Funeral attendees who ate beef stew at the funeral were more likely to develop food poisoning, suggesting that the beef stew was contaminated with *Aeromonas* from the unboiled water.

Public health actions

We disseminated the findings to Jinja District Task Force (DTF), and leaders of the affected communities. Subsequently, the DTF resolved to enhance Water and Sanitation, and Hygiene (WASH) interventions in the affected villages including distribution of chlorine water treatment tablets to all households and replenishing chlorine dispensers at all the water collection points.

Recommendations

We recommended the following measures to control the outbreak and mitigate the risk of future foodborne disease outbreaks.

Stopping the use of water from 'Kabakubya" stream indefinitely, since it was untenable to treat the stream water at source, and yet the village had several alternative safe water sources such as boreholes and pumped water tap systems.

Conducting community sensitization on causes and prevention of food poisoning with emphasis on personal hygiene, sanitation, food preparation and handling practices to prevent secondary cases and the risk of future foodborne disease outbreaks. The sensitization messages should also emphasize the importance of timely health care seeking for those that develop symptoms for favorable treatment outcomes.

Intensifying and sustaining WASH interventions in the affected villages such as provision of chlorine for water treatment, encouraging people to boil drinking water and ensuring proper disposal of feces to halt further transmission of *Aeromonas*.

Refresher trainings for the district and health facility surveillance teams on food poisoning outbreak investigation should be considered. This would facilitate timely detection and proper response in any future outbreaks of a similar nature.

On job orientation of the clinical teams on proper laboratory investigations, and management of food poisoning should be conducted to avoid missed opportunities for identifying the causative agents in case of future outbreaks.

Abbreviations

aOR	Adjusted Odds Ratio
cOR	Crude Odds Ratio
AR	Attack Rate
CDC	United States Centers for Disease Prevention and Control
CFR	Case Fatality Rate
CPHL	Central Public Health Laboratories
DGAL	Department of Government Analytical Laboratory
EDTA	Ethylenediamine Tetra-acetic Acid

ESBL	Extended Spectrum Beta-Lactamase
GCMS/MS	Gas Chromatography-Mass Spectrometry Mass Spectrometry
IQR	Interquartile Range
MoH	Ministry of Health
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time–Of-Flight
QGIS	Quantum Geographic Information System
SC	Sub-county
TC	Town Council
UBOS	Uganda Bureau of Statistics
WASH	Water and Sanitation, and Hygiene
XLD	Xylosine Lysine Deoxycholate

Supplementary Information

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Supplementary Material 1

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Author contributions
YN, IS, DA, BK, SSM, YM, GO, PB, SW, EN and THN, designed the study and contributed to the data collection and analysis. YN led the writing of the manuscript. YN, BK, RM, LB, and ARA participated in manuscript writing and review to ensure scientific integrity and intellectual content. All the authors contributed to the final draft of the manuscript. All the authors have read and approved the final manuscript.

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Data availability
The datasets on which our findings are based belong to the Uganda Public Health Fellowship Program. For confidentiality reasons, the datasets are not publicly available. However, the datasets are available upon reasonable request from the corresponding author with permission from the Uganda Public Health Fellowship Program.

Declarations

Ethics approval and consent to participate
This study was conducted as a response to a public health emergency by the National Rapid Response Team. The Ministry of Health Uganda provided administrative clearance to conduct this investigation. The US Centers for Disease Control and Prevention (CDC) provided the non-research determination (NRD) for non-human subjects. In agreement with the International Guidelines for Ethical Review of Epidemiological Studies by the Council for International Organizations of Medical Sciences (1991) and the Office of the Associate Director for Science, US CDC/Uganda, it was determined that this activity was not human subject research and that its primary intent was public health practice or disease control activity (specifically, epidemic or endemic disease control activity). This activity was reviewed by the US CDC and was conducted consistent with applicable federal law and CDC policy. §§See, e.g., 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. § 241(d); 5 U.S.C. § 552a; 44 U.S.C. § 3501 et seq. All experimental protocols were approved by the US CDC human subjects review board (The National Institute for Occupational Safety and Health Institutional Review Board) and the Uganda Ministry of Health and were performed in accordance with the Declaration of Helsinki. Furthermore, verbal informed consent was obtained from all the participants and from the legal guardians for the participants who were below 18 years of age for interviews since the investigation presented no more than minimal risk of harm and involved no procedures for which written consent is normally required in other contexts. Additionally, verbal consent opted for in an effort to minimize possible transmission of COVID19 and other communicable diseases during the investigation. We conducted the interviews in privacy to ensure confidentiality and the data kept under password protection by the study team.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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