RESEARCH ARTICLE

Investigation of a cluster of acute-onset seizures and deaths among children, Sirohi District, Rajasthan, April 2022

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Abstract

Background: Following reports of seven deaths among children with seizure and vomiting in the village of Sirohi, Rajasthan in April 2022, an epidemiological investigation was conducted.

Methods: We established a hospital-based surveillance and did active case finding. A case was defined as vomiting and acute onset seizure or altered sensorium in <18 years old in Verafalli village. We conducted verbal autopsy and collected information on food, behaviours and health-seeking. Food samples, cerebrospinal fluid (CSF) and blood specimens were collected for testing biochemical, bacterial and viral markers. Environmental surveys were conducted around cases’ houses for presence of poisonous vegetation and potential vectors.

Results: Among 11 cases (median age 7 years (2-16 years), females 27%), all had vomiting, new-onset seizure was present in 73% and altered sensorium in 27%; 33% were hospitalized. Case fatality was 64%. No new cases were identified after April 2022. Median time between symptom onset and death was 9 hours (2-54 hours). Among deaths 28% (2/7) were hospitalized. Two deaths had low weight-for-age, blood glucose <25 mg/dl (refractory hypoglycemia) and elevated liver enzymes (SGPT >8000 IU/L). Lab results for food and CSF samples were inconclusive for bacterial and viral markers. Toxicology screen of one death was negative for organophosphates. In 25% (2/8), tick pools tested positive for rickettsia. All cases had houses in vicinity or within fennel crop area.

Conclusions: This acute health event cluster presenting as acute seizures with rapid progression among children in a rural setting was likely due to environmental toxin consumption; high fatality may result from uncorrected metabolic derangement. Aflatoxin is commonly known to infect fennel crops. We recommend early identification and case management to prioritize metabolic derangement correction; continued surveillance and a systematic epidemiological investigation to evaluate the role of environmental toxins particularly aflatoxin as the underlying etiology for similar events in future.

Keywords: Outbreak, Acute Encephalitis Syndrome, Aflatoxin, Poisoning

Introduction

Acute onset seizures in children can be a manifestation of bacterial, viral, or toxicological pathology[1-3]. In children below five years of age febrile seizures and epilepsy can be a common reason for such seizures[4-6]. However, in many cases, the exact cause can remains unknown[1]. Acute Encephalitis Syndrome (AES) is a broad terminology used in epidemiologic surveillance which can present as seizures or convulsions or altered sensorium with fever in children[7].

In the Indian context, Japanese Encephalitis (JE), dengue, and scrub typhus are a few of the major causes contributing 15-20% of AES cases in children. However, still in nearly three-fourths of cases, the diagnosis remains unknown[8]. These unknown causes can be from toxic substances or drugs or consequent to acute hepatic injury[9-12]. The incidence of AES in India ranges from 2.3 to 15.3 cases per 100,000 population, with an average of 7.6 cases per 100,000 population[13-15]. In India AES is reported from 23 states with numerous outbreaks being reported from various districts of West Bengal, Odisha, Uttarakhand, Uttar Pradesh, Maharashtra, Andhra Pradesh, and Bihar with cases from other areas[13,14,16-21]. These areas also have weak healthcare services with overburdened facilities at the field level hence, in the absence of diagnostic facilities, the exact etiology can remain unknown.

While a diagnosis of viral and other etiologies like the Chandipura virus, JE, Dengue, etc. can be done easily, the exact cause of AES can remain elusive in the outbreaks or cases where the underlying cause is a toxin[10,22-30].

In April 2022, the State surveillance cell was alerted by the media reporting of the death of seven children from a single village in the Sirohi district of Rajasthan with symptoms of seizure and vomiting with altered consciousness. An epidemiological investigation was carried out under the public health department to confirm the presence of an outbreak, describe the spatiotemporal distribution of cases and provide recommendations to prevent future outbreaks.
Methods

**Study Setting:**
Sirohi district lies in the southern part of Rajasthan state in India, about 300 meters above sea level. The district is one of the hilly regions of the state and borders the state of Gujrat on its southern side. It has a population density of around 202 persons/sq.km. Sirohi is inhabited by tribes such as “Garasia”, “Saharia”, “Bheels” and “Gametis”, the total tribal population of the district is nearly 28%. It is divided into five administrative blocks (Reodar, Sirohi, Pindwara, Abu Road, and Sheoganj).[31] The seven deaths were reported from the Verafali village of Pindwara block in Sirohi district.

**Outbreak confirmation:**
We obtained data of seizure disorders, heat stroke and other syndromes which can cause seizures in children from the concerned Primary health care hospital (PHC) catering to Verafali village and also from Sirohi District hospital for the past two years (2020-21) and compared it with the cases from the present year.

**Case definition and investigation:**
We defined a case as “any resident of Pindwara block of Sirohi who is aged less than 18 years of age presenting with vomiting and acute onset seizure or altered sensorium from 1-17 April 2022”. We formed 10 teams of two field healthcare workers in each team who went from house to house in the village to look for cases, medical camps were also organised in the area to look for more cases possible in the community. Private hospitals were also alerted to report any cases fitting the definition.

**Interviews:** Thorough in-depth interviews were conducted with a case or a caregiver parent (in case the child had died). We took a detailed history of possible exposures, food history and precise time of symptoms onset using a semi-structured questionnaire. To minimize recall bias we cross-checked the information provided during interviews with hospital and lab records where available and physician interviews.

**Laboratory investigation:** We collected available blood and cerebro-spinal fluid (CSF) samples of the deceased and cases for bacterial and virological testing at the District Medical College. Serum samples from close contacts were also sent to the National Institute of Virology (NIV) Pune for testing toxins. Tick samples from the cattle in the houses of the deceased and cases were sent for testing for rickettsia to NIV Pune.

**Food samples:** Samples from the “chuski” (popsicle) production unit and distribution centres were collected and sent to the state Food testing lab.

**Human viscera samples:** Viscera samples of the deceased were Sent to SN Medical College Jodhpur for histopathological examination.

**Environmental investigation:** A thorough survey of the environment and housing condition of the deceased and cases was done. Presence of sanitation facilities, drinking water supply, open drains and the nuisance of sandflies and mosquitoes were checked for in the survey, areas near the houses of the deceased and cases were visited for the collection of sandflies. A viscera sample was also sent for examination. An environmental survey was also conducted to check for the presence of any toxic plants amongst common crops in the neighbourhood that were recently harvested for the season.

**Literature review:** We also carried out a literature search in PubMed using the keywords “Acute Encephalitis Syndrome”, “Aflatoxin Poisoning”, and “accidental poisoning AND Children” to find the case reports of any similar illnesses and the possible etiological agents mimicking the symptoms of the illness under investigation.

**Ethical Approval**
Outbreak investigation was carried out with the purpose to identify the source of contamination for immediate control of the outbreak and to prevent deaths as part of public health response by the Rajasthan State Health Department. Ethical approval is therefore exempted and not applicable as part of public health response. The field response does not involve any human laboratory sample collection for research purposes, and there is no invasive investigations or medical interventions /experiments carried out. All ethical principles and guidelines by the government of India were adopted during the outbreak response.

![Figure 1: Epidemic Curve of the cases, AES outbreak Sirohi, Rajasthan: April 2022](image-url)
Results

District hospital reported seizure cases in 2021 but no death with similar symptoms was reported in the past two years. The PHC had not reported any cases or deaths related to similar illnesses in the last two years. In April 2022 seven deaths in children clustered in time and place (Verafali village) confirmed the outbreak.

We surveyed a total of 543 households with 2720 residents and were able to identify 11 cases including seven deceased. The overall attack rate was 0.4% and the attack rate in the population under 18 years of age was 1%. All these cases were reported from a single village in Verafalli of Pindwada block. The case fatality rate was 64%.

All the cases were from rural households and lived within a radius of two kilometres (1.2 miles). The male-to-female ratio was 8:3 with a median age of 7 years (range 2-16 years). The median time difference between the onset of symptoms and deaths was 9 hours (2-54 hours). Seizures and vomiting were seen in all the cases while altered sensorium (presented with a history of talking incoherent and non-sensical speech) was seen in three cases. Fever was present in 5 out of 11 cases. (Table 1)

We took a detailed history of the cases for possible exposures (Table 2). There was a history of eating locally made popsicle called “chuski” by three out of eleven cases however apart from that, there was no history of any consumption of any food from outside any shop, social gathering or any food outlet. However, the parents did not deny the possibility of children eating any vegetation or plant product near the house. The popsicles were made and sold within the village and it was eaten one day before the development of symptoms. These popsicles were manufactured locally using water, flavouring agents and sweeteners, the content of which was not mentioned on the packs.

We explored the possibility of encephalitis due to the Chandipura virus (CHPV) and scrub typhus carried by sandflies and ticks on cattle and found that all the cases had cattle within the house premises and had direct or indirect exposure to them. We also took a house made up of only bamboo, grass, mud, cow dung and straws (kachha house) as a risk factor since these kinds of houses favour the population of sandflies[32].

We also explored the possibility of heat stroke and observed that only two of the cases had any possible exposure to heat while playing outside the house.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Deceased (n=7)</th>
<th>Other Cases (n=4)</th>
<th>Total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>New onset seizure</td>
<td>7 (100%)</td>
<td>1 (25%)</td>
<td>8 (73%)</td>
</tr>
<tr>
<td>Altered Sensorium</td>
<td>3 (43%)</td>
<td>0 (0%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (100%)</td>
<td>4 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>1 (14%)</td>
<td>2 (50%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>Fever</td>
<td>2 (29%)</td>
<td>3 (75%)</td>
<td>5 (45%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Deceased (n=7)</th>
<th>Other Cases (n=4)</th>
<th>Total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popsicle</td>
<td>3 (43%)</td>
<td>0 (0%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>Cattle</td>
<td>7 (100%)</td>
<td>4 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Kachha House</td>
<td>1 (14%)</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Heat Exposure</td>
<td>2 (29%)</td>
<td>0 (0%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>Outside food consumption</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Wild plant fruit</td>
<td>1 (14%)</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
</tr>
</tbody>
</table>
Results of Environmental and Entomological Investigation

The areas near the cases showed the presence of wild and poisonous plant of Cassia occidentalis (known as “aavli” in the local dialect), which is used as a remedy for knee pain. Fennel (Foeniculum vulgare) and castor bean (Ricinus communis) crops in the vicinity had been harvested recently. There were no open sources of water near the houses or any open sewage disposal. Sand flies were absent from the houses of all the cases, while eight pools of tick samples were obtained from the cattle present in the houses.

Clinical laboratory test results

Due to the paucity of samples, we were able to access the blood reports of two cases from the hospital, one of whom died before the investigation, the blood glucose level was less than 25 mg/dl in both cases indicating severe hypoglycemia. The serum ALT (alanine aminotransferase) level of both cases was elevated to a level of more than 7000 U/L indicating liver failure.

We were able to get the test results of two CSF samples taken from the case and death. Human Herpes Virus 7 (HHV 7) was found in trace quantity in the sample of CSF of the deceased while the other CSF sample tested negative for all the bacterial and viral markers. The sample sent for the presence of common toxins in the serum sample tested negative for all the common toxins prevalent in the area (organophosphorus, drugs, opioids).

We sent five samples of the “chuski” (country-made popsicle) to the food testing lab at the medical college in the state. Three samples were taken from the production unit and two from the point of sale in the village. Out of all the samples, one sample tested positive for Acinetobacter baumannii. We were also able to collect eight pool of tick samples from the cattle from the houses of the cases, one out of these eight samples tested positive for Rickettsia. The viscera sample was declared contaminated by the lab and the result was not obtained.

We considered the possible causes which can have the findings similar to our present study and tabulated the results. We made the list of possible aetiologies based on the limited samples, data and reports available and narrowed the diagnosis to either some infective or non-infective cause or accidental poisons or toxin ingestion (Table 3). In the table below the positive sign indicates the common finding in both this outbreak and the cause mentioned while the negative sign indicates that the finding did not match the cause mentioned.

Based on the findings from the lab, environmental and clinic-epidemiological data, toxin or poisonous cause was inferred as most potential reason of the outbreak, a thorough literature search was done to ascertain the singular toxin or poison responsible for the symptoms and lab findings in the present cluster of cases. We tabulated the characteristic findings from the present outbreak and matched them with the possible poison or toxin exposure found in the literature search (Table 4).

Table 3: Possible aetiology based on the finding

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Toxin/Poison</th>
<th>Infective</th>
<th>Non-infective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute onset</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Altered sensorium</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hepato-encephalopathy</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Refractory hypoglycaemia</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Exposure potential in present outbreak</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pathogen isolation</td>
<td>NA</td>
<td>-</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 4: Possible poisonous or toxin having characteristics of illness under investigation[24,27,33–45]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Aflatoxin</th>
<th>Cassia</th>
<th>Pesticides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiefly affecting children</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Severe hypoglycaemia</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acute hepatoencephalopathy</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Seizure</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fennel Crops</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Harvesting season</td>
<td>NA</td>
<td>-</td>
<td>NA</td>
</tr>
</tbody>
</table>
Discussion

Acute encephalitis syndrome is a public health problem in India and has known to cause outbreak in various states\cite{16,17,19,21,40}. In the resource crunch settings, it becomes difficult to extract the exact cause and our present investigation is one such example. We started the investigation based on the preliminary media alerts generated and based on the initial investigations we considered this as an outbreak of AES. Since the death had already occurred before the initiation of investigation we faced challenges during the investigation. We had to rely heavily on the verbal autopsies and laboratory investigations. Most of the cases did not get admitted to the hospital and only two out of seven deaths which happened were admitted to the hospital. We accessed the lab reports from the facilities which catered to these two cases. Based on the findings from the labs and the field we were able to narrow down the diagnosis to some extent.

As can be inferred from Tables 3 and 4, we matched the clinical and epidemiological findings with the characteristics of possible infective and non-infective causes. The present outbreak had affected only children aged less than 16 years with acute onset of altered sensorium or seizure, which might be reflective of hepatoencephalopathy as evident from the laboratory results of the two cases whose lab reports were available. Fever was present in nearly half of the cases which pointed towards an infective aetiology. However the median duration between the onset of symptoms and death was only nine hours which with absence of corroborating laboratory findings in which all the bacterial and viral marker tested negative ruled out infective cause.

In the infective causes we considered Chandipura Virus (CHPV) as a possible pathogen. It is known to cause an outbreak of AES in the neighbouring state of Gujarat in the area which lies close to the border of Sirohi. CHPV has high fatality rate of more than 50% which is seen in this outbreak\cite{20,45–48}. Also presence of vegetation near the house holds of the cases which is a contributing factor for the presence of sandflies which are a known carrier of the virus made our suspicion prominent\cite{49,49–52}. However the absence of sandfly on environmental investigation along with laboratory investigation of CSF tested negative for all these infective pathogens as well as other bacterial and viral pathogens.

The presence of vomiting in all the cases consolidated our view towards toxins. We directed our search towards the possible toxins which can cause the observed symptoms and with the propensity to affect children. While Ricinus communis, if eaten, is a cardiac poison and because cardiac symptoms could not cause the observed symptoms\cite{53–56}, poisoning from pesticide was considered as a possible cause. We considered the organophosphorus (OP) as possible culprit as it is generally used in Sirohi district for agricultural purposes. The OP poisoning can affect all ages and is not exclusively seen in children. The typical symptoms of OP poisoning like salivation, lacrimation, urination, defecation, gastric cramps, emesis (SLUDGE), were not present in our cases (except vomiting) \cite{34,35,57–59}. Furthermore timing of the cases occurring in harvesting season, when pesticides are not used, made the pesticide an unlikely cause for the outbreak\cite{34,35,57–60}.

There have been cases in the past of Senna articulata or Cassia occidentalis, which are plants of the same family, poisoning in children, especially in rural areas of India; it has medicinal properties and is used for alleviation of joint pain amongst tribal and rural population.\cite{61–63} The plant is found in abundance in these areas. The green vegetative part of the plant contains beans and its known to cause similar symptoms in children\cite{10,12,25,26,64}. We also found these plants in the surrounding areas of the deceased and suspected it as one of the causes of the outbreak, however, due to the absence of any toxicology lab in the state we could not conclusively affirm its role.

Aflatoxin is the only toxin which causes similar symptoms and the deaths are mainly seen in children with presence in the harvesting season of fennel crops. The fungus is known to cause these symptoms and can infest the harvested and stored crops of maize, wheat and fennel. Children have access to a large quantity of fennel within and around the household during and after the harvesting season, parents also said that children used to eat surrounding vegetations during cultivation and harvesting season, hence there is a strong possibility of ingestion of aflatoxin-infested fennel when they were not supervised by any adult family member\cite{24,33,37,40,41,43–45,65,66}.

In the present outbreak we had few resources at our disposal for testing, as the deaths had already occurred and the bodies of the deceased had been cremated. There was also no toxicology lab in the district and the viscera samples which were sent to the forensic lab was delayed and mishandled which resulted in inconclusive test results. However based on the detailed history and verbal autopsy, along with available clinic-pathological reports we were able to narrow our diagnosis to aflatoxin toxin ingestion by the cases.

The outbreak occurred in a rural location with an abundance of agricultural land around the cases, with the presence of acute onset and rapidly progressing hepatoencephalopathy which made poisoning a strong possibility. Storing food grains for long durations can be associated with aflatoxin production and subsequent poisoning following consumption, which is more prominent in children. We ruled out possible causes through thorough investigation and based on the field observations along with clinical and lab findings reached a conclusion that Aflatoxin infestation of the harvested fennel crops can be the causal factor for this outbreak\cite{24,33,37,40,41,43–45,65–67}.

Limitations

The present investigation had limitations as there was a paucity of samples from the cases and the testing facilities were also limited, however, we were able to direct our investigation by ruling out the possible causes
to reach a plausible conclusion. There was also a limitation as the data is mainly obtained from the verbal autopsy, leading to potential recall bias, however, we triangulated the data from other sources to increase reliability.

**Recommendations**

As the median incubation period between the disease and the death was very low with clinical findings of hypoglycaemia, we recommend strengthening public health infrastructure including training of the field level healthcare workers in identifying AES cases timely and management of hypoglycaemia to be started as soon as possible in such cases. We also recommend the state health department establish public health laboratory facilities at the district level. Additionally, a district surveillance unit to enhance preparedness, ensuring efficient response to AES outbreaks, with a surveillance focus on rural areas and agricultural communities vulnerable to toxin-related incidents. We would also recommend that the staff at the facility or district level should also be trained in the collection and handling of viscera samples as the contamination of samples can thwart all the efforts taken to establish the cause of an outbreak or disease.

**Conclusion**

This acute health event cluster presenting as acute seizures with rapid progression among children in a rural setting was likely due to environmental toxin consumption; with the high fatality rate possibly resulting from uncorrected metabolic derangement. Aflatoxin is commonly known to infect fennel crops. We recommend early identification and case management to prioritize metabolic derangement correction; continued surveillance and a systematic epidemiological investigation to evaluate the role of environmental toxins particularly aflatoxin as the underlying etiology for similar events in future.

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