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Research Note

An Investigation of Bongkrekic Acid Poisoning Caused by Consumption of a Nonfermented Rice Noodle Product without Noticeable Signs of Spoilage

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ABSTRACT

Bongkrekic acid (BKA) is a tricarboxylic fatty acid that inhibits adenine nucleotide translocase as a kind of mitochondrial toxins. BKA is produced by the bacterium *Burkholderia gladioli* pathovar *cocovenenans*. An investigation was performed to determine the source of possible BKA poisoning of a family in H City, Guangdong Province, People's Republic of China, who consumed a commercially produced rice noodle product that was not fermented or noticeably spoiled. Clinical and food samples were tested. BKA concentration was detected by liquid chromatography–tandem mass spectrometry. We isolated and identified the suspicious strains from the rice noodles and performed toxicity determination through an animal experiment. BKA detected in the cases and the dead dog was 2.15 to about 343 μg/kg. The cases and dead dog shared a unique history of food exposure. The BKA in the factory's food samples was 150 and 160 μg/kg. All mice given the BKA extract by gavage died within 24 h. In conclusion, the food poisoning was caused by the high BKA concentration of expired (4 days over the 24-h shelf life) wet rice noodle products, with corn and wheat starch contaminated by *B. gladioli cocovenenans*. Different from traditional BKA poisoning caused by fermented and spoiled corn or coconut products, there was no noticeable spoilage because of the nonfermentation process and overused sodium dehydroacetate. The risk of BKA in wet rice noodle products and application of antiseptics, such as sodium dehydroacetate, in such food should be quantitatively evaluated to prevent the recurrence of similar events.

HIGHLIGHTS

- Wet rice noodle products (WRNPs) contaminated by *B. gladioli cocovenenans* can produce BKA.
- WRNPs were unfermented without spoilage, which differs from previous BKA poisoning.
- Preservative addition inhibits food spoilage but not BKA production.

Key words: Bongkrekic acid; *Burkholderia gladioli* pathovar *cocovenenans*; Fermentation; Sodium dehydroacetate; Spoilage; Wet rice noodle products

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Bongkrekic acid (BKA) is a toxin produced by *Burkholderia gladioli* pathovar *cocovenenans (12)* (also known as *Pseudomonas cocovenenans* subsp. *farinofermentans* in the People's Republic of China). There are four main categories of foods that are susceptible to being contaminated by BKA: completely or incompletely fermented corn and coconut products; spoiled, fresh *Tremella fuciformis* Berk.; and other spoiled starchy products (glutinous rice, millet, sorghum rice, and potato flour, etc.) *(4)*. As early as 1960, *P. cocovenenans* subsp. *farinofermentans,* a type of foodborne pathogen, was extracted from tempe bongkrek (spoiled food produced with coconut milk and beans) in Indonesia *(19)*. Before 2015, reported food poisoning caused by this bacterium occurred only in China and Indonesia in Asia *(1)*. Indonesia has reported a cumulative number of more than 9,000 people have been affected, with more than 1,000 deaths resulting mainly from spoiled and fermented coconut products since the end of the 19th century. Since the mid-20th century, China has reported a cumulative number of more than 9,000 affected people, with more than 1,000 deaths primarily from spoiled and fermented corn products. In 2015, a poisoning event from a fermented and spoiled cornmeal beverage was

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reported in Mozambique, Africa (230 people affected, 75 dead) *(13)*. Therefore, high-risk food causing BKA poisonings in the past have mainly been a result of fermented or spoiled corn or coconut products.

Among more than 60 species of *Burkholderia* bacteria, only *B. gladioli cocovenenans* produces BKA (highly toxic) and toxoflavin *(23)*. Optimum toxin-production temperature is between 26 and 28°C. In semisolid potato-dextrose agar medium at 26°C, BKA starts to be produced on the second or third day and reaches its peak on the fifth day *(19)*. BKA is a highly unsaturated tricarboxylic fatty acid, which is poorly soluble in water and is soluble in various organic solvents. It is a heat-stable and fat-soluble substance, whose production depends on the presence of fatty acids, particularly those found in coconut and corn *(1, 15)*.

BKA produces its toxic effects by inhibiting mitochondrial adenine nucleotide translocase *(3, 21)*. The latency period for BKA is reported to be 2 to approximately 24 h. An early symptom of BKA poisoning is gastrointestinal stimulation. The disease progresses rapidly, leading to failure of multiple organs, such as the brain, liver, and kidney; death rates range from 40 to 100%, depending on exposure rate *(4, 17)*. In October 2018, a family poisoned by BKA, which was suspected to have been caused by an expired wet rice noodle product, was reported in Guangdong Province, China. The poisoned food was neither fermented nor noticeably spoiled. In this study, we performed investigation and analysis to clarify the causes of the poisoning and the production process of the food to explore possible risk factors and preventive control measures.

MATERIALS AND METHODS

Materials. This food poisoning incident was found by a local district center for disease control and prevention and submitted to the municipal and provincial centers for disease control and prevention. These institutions organized technicians to perform field investigations and laboratory tests to collect the epidemiological and clinical results.

Methods. A field epidemiological investigation was performed. The suspicious food-exposure history of the case was analyzed, and a food hygiene investigation was conducted. The inpatient medical records of the case and the deceased were collected to analyze their clinical characteristics by interviewing doctors and family members of those involved in the incident. Blood, urine, vomit, and the contents of a dead dog's stomach were collected as was the same batch of rice noodles. Based on the clinical manifestations and research results, rodenticides, toadstools, and heavy-metal poisoning were excluded. We performed testing on samples and detected the presence of BKA, according to the "National Food Safety Standard and Determination of Bongkrekic Acid in *Tremella fuciformis* Berk" (GB5009.189-2016) *(7)*. According to procedures in "Microbiological Examination of Food Hygiene—Examination of *Pseudomonas cocovenenans* subsp. *farinofermentans*" (GB/T 4789.29-2003) *(5)*, we isolated, cultured, and identified the bacterial strains in the noodle samples and performed mice experiments by giving the crude extract from the toxin of the cultured bacteria intragastrically to determinate its toxicity. Based on "Epidemiological Investigation of Food Safety Accident Technical Guidance" *(11)* and "Diagnostic Criteria and Principles of Management for *Pseudomonas cocovenanans* subsp. *farinofermentans* Poisoning" (WS/T 12-1996) *(4),* we synthetically analyzed the poisoning accident, combining all of the resources above.

RESULTS

General information. The family involved included seven people: a grandpa, grandma, father (25 years old), mother, an eldest son (3 years old), a second daughter (2 years 5 months), and the youngest son (1 year old). The family lived on a remote island in the town of H City, Guangdong Province. The island is surrounded by a river, and the local temperature was about 24 to about 26°C, with a warm and humid climate at the time of the incident. On the afternoon of 19 October 2018, the father bought about 3 kg of wet rice noodles for breakfast for the next 4 days (the family ate at about 6:00 a.m.). On 23 October 2018, at 6:00 p.m., the dog was fed the rest of the noodles from early that morning. The onset of symptoms for the father and mother was at 8:00 a.m. on 23 October 2018, and the second daughter and the eldest son became ill at 1:00 p.m. and 3:00 p.m., respectively, with symptoms of diarrhea, vomiting, coma, convulsions, and other symptoms. The second daughter and her mother died in the hospital from multiple organ failure. The father was in intensive care, but the eldest son did not seek medical treatment. The dog was found dead on the night of 25 October 2018.

Epidemiological investigation. The onset of the disease was familial aggregation. The breakfast intake of the seven people was about 300 g for adults, 150 g for the eldest son, 100 g for the second daughter, and 50 g for the youngest son (with breast-feeding). No peculiar smell was found in the process of cooking and eating the wet rice noodles; there were no color and character changes, and no taste abnormalities were observed. The amount of leftover wet rice noddles eaten by the dog is unknown.

The sanitary condition of the family was poor and the humidity was high. Humans and the animal (a dog) lived in the same room. They drank mountain spring water in their daily lives. The rice noodles were stored open at room temperature (24 to about 26°C). The production process of all 4 days of breakfast remained the same: the rice noodles were rinsed with clean water, the water was brought to a boil, the rice noodles were put into the water, the cook waited for the water to return to a boil, and then, the noodles were fried with pork, and condiments were added at the end.

On 19 October 2018, the family bought the noodles at a vegetable store. The food was produced by X food factory of Z city on the evening of 17 October 2018 and was distributed intercity by dealers to the X vegetable store of H city on the evening of 18 October 2018. The X food factory produces more than 50,000 kg of rice and noodle products every day, and the shelf life is marked as 24 h. The factory's production techniques were to wash the rice in cold water and allow it to soak (for about 45 min), grind the rice into a thick liquid in a machine, mix the rice slurry with wheat and corn starch (rice/wheat starch/corn starch ratio = 18:6:1) using high-pressure steam injection, allow it to cool, cut it

TABLE 1. *BKA concentrations in four different case samples and a dog*

Cases	Sample types	Concn
The second daughter (dead)	Vomitus (tissue wiping)	2.15 μg/kg
Mother (dead)	Blood	154 μg/L
	Urine	2.77 μg/L
	Liquid of gastric lavage	1.04 μg/L
Father (severe patient)	Blood	137 μg/L
	Urine	50.8 μg/L
	Vomitus	37.9 μg/kg
The eldest son (not hospitalized)	Blood	2.59 μg/L
	Urine	$<$2 μg/L
Dog (dead)	Gastric contents	343 μg/kg

into pieces with a little soybean oil to prevent adhesion, and then package the product. Sodium dehydroacetate (DHA-S)—a type of food preservative—was added to the process during the soaking of the rice (45 min, about 100 g of DHA-S per 450 kg of rice). The factory has no cold storage. The process of storage, distribution, and sales of finished products were performed at room temperature.

Clinical manifestation. Dizziness, nausea, vomiting, abdominal distension, pain, and fatigue were symptoms that appeared in all four cases, with no fever. The most severe symptoms included dyspnea, disturbance of consciousness, convulsion, yellow skin, coma, multiple organ failure including the kidney and heart, and death. An auxiliary clinical examination showed all patients had liver function damage and abnormal coagulation functions. After gastric lavage, emetic stimulation, and detoxification treatment, two of the patients died; one patient with severe poisoning was successfully rescued and was discharged from the hospital on 9 November 2018.

Laboratory detection. BKA detection was performed on the clinical samples from six people (excluding the second daughter) and on the stomach contents of the dog. The results showed different concentrations of BKA in blood (six samples), urine (six samples), vomitus (two samples), liquid from the gastric lavage (six samples) of the four family members, and the dog's stomach contents (Table 1).

Wet rice noodle products by the same factory produced during the same period and preserved under the same conditions were collected. After 48 h of exposure to 26°C temperature at 50 to about 90% humidity, the concentrations of BKA in two noodle samples were 150 and 160 μg/kg.

After strain isolation and cultivation, the clinical and 16S rDNA test results showed that the BKA-producing strains were *Burkholderia gladioli* pathovar *cocovenenans*. In the toxicity test, mice were given the crude extract of the BKA from the cultivated supernatant of the wet rice noodles. All mice died, with hepatocyte necrosis, vacuolar deformation, and thrombus congestion.

DISCUSSION

In this incident, four poisoned family members and their dog had the same history of food exposure—all had consumed expired, wet rice noodle products (24-h shelf life) before the onset. The clinical manifestations among the patients were similar, primarily vomiting, diarrhea, and liver damage, which are consistent with prior BKA poisoning incidents. Approximately 2.15 to 343 g/kg BKA were detected from the clinical samples, and 150 and 160 g/kg BKA were found in the noodle samples. It was determined that this was a family incident of BKA poisoning caused by the consumption of expired, wet rice noodle products with added DHA-S, with the investigation and test results excluding other possibilities *(4)*. There was no fermentation process in the production of the wet rice noodle products and no obvious signs of spoilage in the food, so we assumed several possible risk factors.

First, BKA is produced by *B. gladioli cocovenenans*. Wet rice noodle products contaminated by *B. gladioli cocovenenans* in the factory could produce BKA after improper storage (expired storage at room temperature, generally at least 2 days over the shelf life at about 24 to 28°C). The raw material of the wet rice noodle products (rice, wheat starch, and corn starch) are easily contaminated by *B. gladioli cocovenenans* in the process of soaking, grinding, and cutting. In this incident, the wet rice noodle products may have been contaminated by bacteria at the time of shipment. The noodles were stored for more than 5 days (2 days before being sold and 4 days of eating) at room temperature (about 24 to 26°C), which provided an adaptive environment for the virulent production of *B. gladioli cocovenenans*.

Second, the preservative DHA-S was added during the production of the wet rice noodle product. DHA-S is not affected by the pH of the food and has a strong inhibitory effect on yeast, mold, and putrefying bacteria, which often cause food spoilage *(22)*. Anwar et al. *(1)* found that the production of BKA can be inhibited by *Rhizopus oligosporum*, and BKA concentrations can be reduced in the presence of a sufficient number of fungal colonies (complete fermentation) *(2, 14)*. However, there are also reports of *Rhizopus microsporus* increasing the amount of BKA in a stationary medium *(8, 16)*. Therefore, DHA-S added to the production of wet rice noodle products may hamper or facilitate the inhibition of BKA through its effects on mold. Wet rice noodle products have high water activity and are suitable for bacterial growth and reproduction. The use of DHA-S may control mold and other microorganisms and reduce the color, smell, taste, and other sensory properties of food caused by fermentation or spoilage, but it cannot prevent the growth of bacteria and the production of toxins, such as *B. gladioli cocovenenans*. As a result, wet rice noodle products may have no obvious signs of spoilage to the senses, but in fact, they may not be edible. This could increase the incidence of foodborne diseases and lead to great safety risks. DHA-S cannot be used as a food additive according to "General Standard for Food Additives" *(9)*. However, some countries have expanded its range of application. According to Chinese "Food Safety National Standards for the Use of Food Additives" (GB 2760-2014) *(6),* DHA-S is applied in the preservation of starchy products, cakes, bread, cooked meat products, fermented soybean products, pickled vegetables, and other foods, with the maximum usage of 1 g/kg. In Japan, DHA-S is used in butter, cheese, and margarine. In the United States, it is used or intended for use as a preservative for cut or peeled squash. In China, only four kinds of dry, starchy foods are allowed to use dehydroacetic acid and its sodium salt, and wet-powder noodles are not included.

Third, the addition of corn starch (4.0%), wheat starch (24.0%), and soybean oil (at about 0.5 to 2%) into the traditional production formula of wet rice noodle products changes the traditional rice formula in the production process of the Guangdong River flour. It is not clear whether it is possible to make a better culture medium for the toxin production of *B. gladioli cocovenenans*. BKA production depends on fatty acids, especially those found in coconuts and corn, and the yield of toxin reaches its maximum in a growth medium containing oleic acid *(10)*. However, corn and wheat starch cannot support enough special fatty acids. Therefore, it remains to be confirmed whether the amount of soybean oil was enough to stimulate *B. gladioli cocovenenans* and BKA production. In China, there are no local or industrial standards to regulate the raw materials and formulae of wet rice noodles. Whether corn and wheat starch and soybean oil can be added into wet rice noodle products and the proportions of addition is worthy of further study and deliberation. BKA is a fat-soluble toxin, which is easily distributed unevenly in solid food. Therefore, the other three family members who also ate the wet rice noodle products in this incident had mild or no symptoms.

Reported cases of food poisoning caused by *B. gladioli cocovenenans* have mostly been the result of "fermented food." Rice noodle products can be processed either by fermenting or without fermenting *(25)*. In the traditional fermentation process, it usually takes about 2 to 6 days to finish fermentation during soaking without additives to promote the fermentation *(18)*. In this incident, the wet rice noodles were nonfermented during the production process. Food contaminated by microorganisms (bacteria, molds, yeasts, etc.) will deteriorate because of the metabolites of the microorganisms (a few yeasts will cause food to deteriorate after fermentation, and the main microorganisms causing flour spoilage are *Bacillus* spp. *(20)*). Spoilage manifests as changes in food color, smell, taste, and other sensory properties, such as a putrid sour smell. Microorganisms in food may also produce toxins, so eating spoiled food is an easy means of causing food poisoning *(24)*. In this incident, the sensory properties of the wet rice noodles remained unchanged, which interfered with people's judgment about the safety of the food. The *B. gladioli cocovenenans* contamination and the production of BKA did not cause food fermentation and spoilage. The toxin production time for the bacteria may coincide with the time required for food fermentation and spoilage. We may draw a conclusion that food fermentation or obvious food spoilage was unnecessary for the production of BKA.

Wet rice noodle products, such as river noodles and rice noodles, are popular and specialty foods of high consumption in Guangdong. Zhang et al. *(22)* showed that the per capita daily consumption of wet rice noodle products in Guangzhou was 60.93 g. Excessive use of DHA-S in rice noodle products was found in different parts of Guangdong by the Chinese Food and Drug Administration. DHA-S was added to wet rice noodle products just as it was to dry vermicelli (06.05.02.01 in GB 2760-2014 *(6)*), which means an expanded application of food additives. Although these additions are required for long-term preservation and long-distance transportation of food, there may be certain health risks to the population in expanding the usage of food additives without adequate risk assessments. Therefore, the safety of wet rice noodle products should be highly valued by relevant departments. The government should assess the risks of food additives, such as DHA-S, being added to certain foods and urge enterprises to strictly carry out the new local standards. Relevant departments should perform further research to quantitatively evaluate nonfermented and nonspoiled wet rice noodle products and establish local standards for wet starchy products as soon as possible. Key points, such raw materials, technology, food additives, shelf life, transportation, and management, should be standardized. Food supervision departments should strengthen their supervision of production enterprises making wet rice noodle products, by severely cracking down on the behavior of selling expired wet rice noodle products, popularizing the harm of *B. gladioli cocovenenans,* publicizing food safety knowledge about wet rice noodle products, and providing guidance in the correct selection, storage, and consumption of wet rice noodle products by the masses. As the regions in which BKA intoxication has happened have now increased to three, we wonder whether there are other regions in which BKA poisoning was misdiagnosed because of a deficiency in knowledge about it. Concerns and further work must be global, rather than being confined to Asia and Africa. The growth of the organism and toxin-production details will be explored in the next step.

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