Haff disease is an unexplained rhabdomyolysis that occurs within 24 hours after consumption of certain types of freshwater or saltwater fish (1,2). It was first reported in 1924 in the vicinity of Königsberg along the Baltic coast near Frisches Haff (1–3). Over the next 9 years, an estimated 1,000 persons were affected by similar outbreaks, occurring seasonally in the summer and autumn in this area (3). Although subsequent outbreaks were identified in several other countries, such as Sweden (4), the former Soviet Union (5), Brazil (6,7), Japan (8), and China (9,10), the etiology has not yet been determined. An unidentified heat-stable toxin similar to cyanotoxins or palytoxin, but primarily myotoxic and not neurotoxic, is thought to be the cause of Haff disease (1); however, evidence supporting this hypothesis has been scant.

In July 2016, the number of rhabdomyolysis cases reported to the National Foodborne Disease Surveillance System (NFDSS) in China dramatically increased in Anhui Province compared with previous years. Most of the cases were reported in Wuhu and Ma’anshan, cities in Anhui Province in eastern China. Epidemiologic features were compatible with Haff disease (3,6). Preliminary investigation implicated crayfish as the vector. On August 5, the number of cases surpassed 200, prompting an emergency investigation by the Chinese Field Epidemiology Training Program, together with the Anhui Province Center for Disease Control and Prevention (CDC). The objectives of the investigation were to describe the epidemiologic and clinical characteristics, trace back the implicated vectors, identify possible risk factors, and recommend control measures.

Methods

Case Definition and Finding
We defined a case of rhabdomyolysis as any person with elevation in creatine kinase (CK) value plus clinical manifestations of myalgia or limb weakness (10,11). We defined a Haff disease case as illness in any person with acute onset of rhabdomyolysis after ingestion of freshwater fish or seafood within 24 hours in Anhui Province during June–August 2016. We searched for physician-diagnosed rhabdomyolysis cases from the NFDSS, an internet-based, passive surveillance system for foodborne
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We also reviewed the outpatient and inpatient medical records in hospitals in Wuhu and Ma’anshan during the outbreak period to search for potential rhabdomyolysis cases.

Local Anhui Province CDC staff or Chinese Field Epidemiology Training Program trainees interviewed all rhabdomyolysis case-patients, either in person or by telephone, using a structured questionnaire. Information collected included age, sex, date of onset, disease duration, clinical symptoms, potential risk factors (e.g., food, drugs, alcohol consumption, intense exercise, allergy history, underlying chronic illness), and the quantity of crayfish consumed. The researchers also obtained laboratory test findings from hospital medical records. They applied the Haff disease case definition to rhabdomyolysis cases to identify Haff disease cases and collected blood and urine specimens from Haff disease case-patients for further analysis.

In total, 673 rhabdomyolysis cases were identified in Anhui Province during June–August 2016. Of these, 99.9% (672/673) were compatible with the definition of Haff disease. All but 1 patient consumed cooked crayfish before symptom onset. The patient who did not eat cooked crayfish was a steelworker who had been working in the factory before onset, suggesting his illness might have been caused by heatstroke.

**Traceback of Food and Environmental Investigation**

For all Haff disease cases, we conducted a traceback investigation for the source of the implicated food by interviewing case-patients, restaurant owners, fishermen, and crayfish sellers. We conducted an environmental investigation of potential contamination along the distribution chain or unusual events during the outbreak period. We investigated the restaurants where case-patients had a meal before onset to find out where the crayfish came from and how they were cooked. We also visited crayfish farms, settings where crayfish were caught, and factories along the Yangtze River to identify whether the implicated crayfish or the environment in which the crayfish were raised had been contaminated.

**Data Analysis**

We performed statistical analysis using SPSS Statistics 20 (IBM, https://www.ibm.com). We compared cases and controls by \( \chi^2 \) test. Significant risk factors (p<0.05) in the \( \chi^2 \) tests were included in a multivariate Cox proportional hazard model to determine the odds ratio (OR) and 95% CI for the potential risk factors associated with Haff disease. All of the p values were 2-sided, and p<0.05 was considered significant.

**Results**

**Confirmation of the Outbreak**

In total, we verified 672 Haff disease cases in Anhui Province during June–August 2016. All cases occurred in 7 cities along the Yangtze River in Anhui Province; 83.3% (560/672) of the cases occurred in Wuhu (334 cases) and Ma’anshan (226 cases). We focused our investigation on the cases that occurred in Wuhu and Ma’anshan. Of the 560 case-patients in Wuhu and Ma’anshan, 495 (88.4%) completed the questionnaires; all 495 had consumed crayfish within 24 hours before symptom onset. The epidemic curve suggested a continuing common-source outbreak (Figure).

**Descriptive Epidemiology**

The outbreak started at the end of June, peaked in mid-July to early August, and lasted through August 17. Of the 495 case-patients, 197 (39.8%) were hospitalized; mean length of hospital stay was 7.3 ± 3.2 days. No deaths were reported. The mean age of the case-patients was 38.7 ± 13.5 years; 323/495 (65.3%) patients were female. Although cases were widely distributed in the 2 cities, 87.7% (434/495) were in residents from urban areas close to the
Yangtze River. Each case-patient consumed a mean of 11.6 ± 6.1 crayfish pieces. The mean incubation period was 6.2 ± 3.8 hours.

Clinical Characteristics
All 495 case-patients experienced myalgia that was local or diffuse, involving the back, waist, whole body, neck, limbs, and chest (Table 1). A total of 271/495 (54.7%) experienced muscle weakness. Additional symptoms included brown urine, dyspnea, vomiting, abdominal pain, dizziness, and headache. Symptoms of nerve paralysis and fever were rare. Acute renal failure was not observed.

| Symptoms          | No. cases (%) | N = 495 |
|-------------------|---------------|---------|
| Myalgia           | 495 (100.0)   |         |
| Back              | 241 (48.7)    |         |
| Waist             | 194 (39.2)    |         |
| Whole-body        | 186 (37.6)    |         |
| Neck              | 186 (37.6)    |         |
| Lower limbs       | 111 (22.4)    |         |
| Upper limbs       | 89 (18.0)     |         |
| Chest             | 59 (11.9)     |         |
| Muscle weakness   | 271 (54.7)    |         |
| Brown urine       | 99 (20.0)     |         |
| Dyspnea           | 60 (12.1)     |         |
| Headache          | 49 (9.9)      |         |
| Abdominal pain    | 46 (9.3)      |         |
| Diarrhea          | 32 (6.5)      |         |
| Vomiting          | 29 (5.9)      |         |
| Dizziness         | 25 (5.1)      |         |
| Nausea            | 21 (4.2)      |         |
| Nerve paralysis   | 11 (2.2)      |         |
| Fever             | 1 (0.2)       |         |

Laboratory Characteristics
We reviewed the laboratory test findings of blood and urine for some cases. The mean value of myoglobin was 330.0 ± 121.2 ng/mL, and mean CK level was 5,439.2 ± 4,765.1 U/L. In >80% of the cases, the levels of muscle-type CK and aspartate aminotransferase were abnormally elevated. In addition, 50.0% of case-patients were positive for urinary occult blood and proteinuria (Table 2).

Case–Control Study
In the case–control study, 100% of the 67 cases and 93.3% (101/108) of controls ate crayfish during their shared meal (OR = ∞, 95% CI 0.92–∞). We observed a significant dose-response relationship between the number of pieces of crayfish eaten and Haff disease \( (χ^2 = 29.225; p<0.001) \) (Table 3). Further analysis showed that eating crayfish liver was associated with increased disease risk (OR = 4.0, 95% CI 1.2–12.7).

Traceback and Environmental Investigation
Wuhu and Ma’anshang are located in the middle to lower reaches of the Yangtze River. Crayfish is a popular dish for residents of these 2 cities. Before the Haff disease outbreak, Anhui Province experienced heavy rainfall, which caused the largest flood disaster in decades. Consequently, rain or floodwater was retained in irrigation ditches and detention ponds for an extended time, and the amount of crayfish caught on the shores of the Yangtze River...
or its connected ditches was 5–10 times more during the outbreak period. However, no industrial or chemical contamination along the Yangtze River was reported.

The only common risk factor for all cases was eating crayfish, which were cooked thoroughly. We conducted a traceback investigation of the source for the implicated crayfish in Ma’anshan and Wuhu by interviewing persons in markets, restaurants, fisheries, and settings where crayfish were caught, as well as fishermen; we were able to trace 50.1% (248/495) of the implicated crayfish to their sources. Of these, 96.8% (240/248) were wild crayfish caught on the shores of the Yangtze River or its connected ditches. When we consulted with crayfish biologists, we found that the species of crayfish implicated during this outbreak was Procambarus clarkii.

Public Health Measures

Local governments issued a warning about the dangers of eating crayfish. In addition, public health departments instituted continuous surveillance and investigation of the outbreak.

Discussion

The epidemiologic and traceback investigations of a large outbreak of Haff disease in Anhui Province, China, indicated that all case-patients consumed crayfish within 24 hours before symptom onset; the implicated crayfish were caught on the shores of the Yangtze River or its connected ditches. The case–control study revealed that eating the liver of crayfish was associated with an increased risk for disease; the risk increased as the quantity of crayfish eaten increased.

Table 2. Laboratory test values from cases during Haff disease outbreak in Anhui, China, 2016*

| Variables          | Reference range | Median (range) | Mean ± SD | No. cases | % Abnormal cases |
|--------------------|-----------------|----------------|-----------|-----------|-----------------|
| **Serologic test** |                 |                |           |           |                 |
| Myoglobin, ng/mL   | <25             | 344.3 (25.0–500.0) | 330.0 ± 121.2 | 97        | 100             |
| CK, U/L            | 30–135          | 4,192.0 (165.0–17,470.0) | 5,439.2 ± 4,765.1 | 191       | 100             |
| CK-MM, U/L         | 0–25            | 79.0 (10.0–980.0) | 161.2 ± 165.9 | 104       | 82.7            |
| AST, U/L           | 14–36           | 73.0 (14.0–1,346.0) | 164.6 ± 207.0 | 103       | 84.5            |
| ALT, U/L           | 9–62            | 82.0 (20.0–615.0) | 85.9 ± 88.2 | 101       | 49.5            |
| LDH, U/L           | 313–618         | 684.0 (333.0–8,170.0) | 1,161.0 ± 1,334.9 | 99        | 53.5            |
| Cre, μmol/L        | 62–106          | 63.6 (34.6–108.3) | 66.4 ± 14.5 | 102       | 44.1            |
| Urea, mmol/L       | 2.5–6.1         | 5.7 (2.3–256.1) | 8.1 ± 24.84 | 102       | 32.4            |
| Cl*, mmol/L        | 98–107          | 104.70 (98.20–109.80) | 104.46 ± 2.42 | 97        | 12.4            |
| Ka*, mmol/L        | 3.6–5.0         | 3.95 (3.14–5.17) | 3.97 ± 0.34 | 97        | 10.3            |
| Ca**, mmol/L       | 2.10–2.55       | 2.30 (2.07–2.65) | 2.29 ± 0.13 | 47        | 8.5             |
| Na*, mmol/L        | 137–145         | 139.20 (133.80–144.50) | 139.25 ± 1.9 | 97        | 8.2             |

**Urinalysis**

| Variables          | No. positive results | % Abnormal cases |
|--------------------|----------------------|-----------------|
| Proteinuria        | 9                    | 50.0            |
| Urinary occult     | 10                   | 50.0            |

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CK-MM, muscle-type creatine kinase; LDH, lactate dehydrogenase; Cre, serum creatinine; Ka*, serum potassium; Na*, serum sodium; Cl*, Serum chloride; Ca**, serum calcium.

Table 3. Analysis of probable risk factors associated with Haff disease in case–control study, Anhui, China, 2016*

| Variables               | Cases, N = 67 | Controls, N = 108 | p value | Multivariable OR (95% CI) |
|-------------------------|---------------|-------------------|---------|--------------------------|
| **Sex**                 |               |                   |         |                          |
| M                       | 19 (28.4)     | 48 (44.4)         | 0.033   | Reference                |
| F                       | 48 (71.6)     | 60 (55.6)         |         | 1.6 (0.9–2.7)            |
| **Mean age, y (SD)**    |               |                   | 0.227   | NA                       |
| 37.3 (11.3)             | 39.4 (18.9)   |                   |         | NA                       |
| **Consumption of crayfish (SD)** |           |                   | 0.084   | NA                       |
| No                      | 0             | 7 (6.5)           |         | NA                       |
| Yes                     | 67 (100)      | 101 (93.5)        |         | NA                       |
| **No. crayfish consumed** |             |                   | <0.001  | Reference                |
| 1–9                     | 27 (40.3)     | 84 (77.8)         |         | Reference                |
| 10–19                   | 26 (38.8)     | 12 (11.1)         | 2.4 (1.4–4.2) | Reference |
| >20                     | 14 (20.9)     | 5 (4.6)           | 2.6 (1.3–5.1) | Reference |
| **Ate liver of crayfish** |             |                   | <0.001  | Reference                |
| No                      | 3 (4.5)       | 32 (29.6)         |         | Reference                |
| Yes                     | 64 (95.5)     | 76 (70.4)         |         | 4.0 (1.2–12.7)           |
| **Alcohol consumption** |             |                   | 0.015   | Reference                |
| No                      | 51 (76.1)     | 97 (89.8)         |         | Reference                |
| Yes                     | 16 (23.9)     | 11 (10.2)         |         | 1.6 (0.9–2.8)            |
| **Fish consumption†**  |             |                   | 0.648   | —                        |
| No                      | 64 (95.5)     | 100 (92.6)        |         | —                        |
| Yes                     | 3 (4.5)       | 8 (7.4)           |         | —                        |

*Values are no. (%) except as indicated. NA, not applicable; OR, odds ratio. SD, standard deviation.
†Corrected χ2 value and p value for univariate analysis.
In China, the earliest reported outbreak of Haff disease was in Beijing in 2000 and involved 6 cases (12). An epidemiologic study revealed that all patients ate crayfish before onset, suggesting a link between crayfish and Haff disease (12). Although the literature shows that eating several species of fish, such as buffalo fish (3), salmon (13), freshwater pompano (7), marine boxfish (8), and pomfrets (9), could trigger Haff disease, almost all Haff disease cases in China were associated with eating crayfish (2). In recent years, Haff disease outbreaks have been reported in other cities in China (14–19). These outbreaks prompted the China CDC to conduct a thorough investigation of Haff disease. Crayfish have become a popular seafood for residents in central and eastern China, especially in June–September. Previous studies have reported that Haff disease shows a seasonal pattern, and outbreaks usually occur in the summer and fall months (1,3,10). Although a large Haff disease outbreak caused by eating freshwater pomfret occurred in October 2009 in southern China (9), most crayfish-related outbreaks (10,20,21), clusters (18,22), and sporadic cases (16) occurred predominantly in the summer. Seasonal crayfish harvest and consumption in June–September likely increases the opportunities for exposure, which may partially explain the seasonal pattern of Haff disease in China (10).

The most commonly reported clinical features in this outbreak were myalgia and muscle weakness, as well as abnormal levels of myoglobin and CK. Increased serum myoglobin concentration is the basis for early diagnosis of rhabdomyolysis (23); however, myoglobin concentrations tend to normalize within 6–8 hours following exposure. Thus, the window of opportunity for diagnosis is short (24). Of note, elevated myoglobin concentrations were observed in all case-patients who were tested in this study; this may be due to prompt medical care and timely laboratory testing in the hospital.

Rhabdomyolysis is a common life-threatening syndrome characterized by the injury of skeletal muscle resulting in the leakage of intracellular contents into the circulatory system (25). Patients with rhabdomyolysis usually experience myalgia, muscle weakness, raised serum CK, and brown urine (24). The etiologic spectrum of rhabdomyolysis is extensive, including crush injuries, ischemia, strenuous exercise, extreme body temperatures, drugs, toxins, infections, hereditary causes, and inflammatory or autoimmune muscle disease (23,25). A substantial number of patients may have no cause identified. We found that nerve paralysis and fever were rare symptoms, all crayfish were cooked thoroughly, and no industrial or chemical contamination was identified; therefore, this outbreak was unlikely to have been caused by infectious or chemical etiologies. Diaz et al. reported that an unidentified, heat-stable, algal toxin with primarily myotoxic rather than neurotoxic properties in seafood has been proposed as a cause of Haff disease (1); whether this toxin also exists in crayfish remains unknown.

Although many Haff disease cases have occurred in cities located in the middle to lower reaches of the Yangtze River, the association between Haff disease and crayfish caught from Yangtze River has not been elucidated in the published literature (10,18,20,26). In recent years, 3 other large Haff disease outbreaks have been reported in Nanjing and Tongling, 2 other cities located in the middle to lower reaches of the Yangtze River (10,19–21). The fact that these outbreaks all occurred in the middle to lower reaches of the Yangtze River suggests that crayfish could be their common etiology.

Studies using a mouse model have found that the hazardous substance from crayfish could cause rhabdomyolysis (27,28). This hazardous substance is specific to certain batches of crayfish. A dose-response relationship has also been observed. These findings in laboratory animals were consistent with the results of human epidemiologic investigation (21,28) and with our case–control study findings.

Our study had several limitations. First, because we lacked data on how many persons ate crayfish in the 2 study cities, we could not calculate the attack rates. Second, not all crayfish were traced back to their sources. Third, we were unable to conduct animal experiments to prove causation.

In conclusion, during this outbreak, the risk for Haff disease was associated with eating crayfish along the Yangtze River. The etiology of Haff disease remains elusive due to lack of knowledge of the underlying disease mechanism of rhabdomyolysis. Our findings might help researchers isolate the toxin that causes this disease.

Acknowledgments
We acknowledge the contributions of all participants of the outbreak investigation, especially public health workers from Wuhu and Ma’anshan.

About the Author
Dr. Ma is an epidemiologist and the director of the Chinese Field Epidemiology Training Program, China Center for Disease Control and Prevention. Her research interests include emergency outbreak investigation, epidemiologic, and surveillance projects.
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