First Reported Outbreak of Abdominal Angiostrongyliasis

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Human abdominal angiostrongyliasis is a potentially fatal disease caused by *Angiostrongylus costaricensis*, a nematode that infects several species of rodents (e.g., cotton rats), causes human abdominal angiostrongyliasis in Latin America and the Caribbean [1–12]. One case that may have been caused by *A. costaricensis* was reported from Zaire [13]. Although the infection is enzootic in Texas [14], the only case of autochthonous human infection that has been reported in the United States [15] was later identified as a case of anisakiasis [16].

In rodents, adult worms live in the mesenteric arteries of the terminal ileum and cecum [17–19]. First-stage larvae are excreted in the feces and ingested by mollusks (e.g., slugs), the intermediate hosts. Rodents become infected by ingesting mollusks that contain infective third-stage larvae, raw food (e.g., produce) contaminated with mucous secretions from infected mollusks [20], and possibly carnivorous planarians that feed on dead mollusks (as was described for *Angiostrongylus cantonensis*, which causes eosinophilic meningitis in Southeast Asia and the Pacific islands [21]).

The main vehicles of transmission to humans, who are accidental hosts, are unknown, as is the incubation period in infected humans; limited data suggest that it ranges from ~14 days [22] to several months [12] and possibly to >1 year [23]. Patients typically have clinical signs resembling those of acute appendicitis and sometimes have intestinal obstruction, perforation, or bleeding [9, 23–25]; some patients are ill for months with relapsing episodes of abdominal pain [25]. The proportion of cases that are oligosymptomatic or asymptomatic is unknown [25, 26]. The case-fatality rate among patients with symptomatic cases ranges from 1.8% to 7.4% [24, 25]. No drug regimen for treating abdominal angiostrongyliasis has been identified, and treatment with some antiparasitic drugs could worsen the course of the illness [27–29].

Clinical cases usually are diagnosed postoperatively by review of surgical specimens. Most cases involve the intestine, especially the ileocecal region, but other organs can also be involved (e.g., liver and testes) [9, 22, 24, 30–33]. Inflammatory response to the adult worms, eggs, and larvae can be severe, with massive eosinophilic infiltration of the intestinal wall, eosinophilic vasculitis, and granulomatous reaction [24, 34]. Distinguishing features include the characteristic microanatomy of the adult worm and its location in the mesenteric arteries, the presence of both eggs and larvae in the intestinal wall, and the size of the eggs (~65 × 40 μm) [12, 17].

In Central America, most reported cases have been in children, predominantly boys, and have been diagnosed during the rainy season [4, 9, 24, 30–32, 35]. From 1 January through 15 April 1995, six cases of abdominal angiostrongyliasis in five adults and a 9-year-old boy were reported to the Guatemala Ministry of Health (Guatemala City), whereas only about one case per year had been reported in recent years. Three of the cases were associated with a series of business luncheons that...
were hosted by one company and catered by several contractors. We began an investigation on 26 April 1995 to identify risk factors for illness. To our knowledge, no outbreaks of abdominal angiostrongyliasis have previously been reported in the literature.

Methods

Epidemiological Investigation

Case definition and case finding. We defined a confirmed case as a patient who had histopathologically confirmed abdominal angiostrongyliasis (i.e., identification of adult worms in mesenteric vessels or of larvae or eggs in the intestinal wall). A probable case was defined as a patient with both histopathologic findings consistent with *A. costaricensis* infection (e.g., massive eosinophilic infiltration with granulomatous reaction in the intestinal wall [34]) and a serum specimen reactive to *A. costaricensis* antigen (see below). A possible case was defined as a patient with clinical symptoms or signs consistent with abdominal angiostrongyliasis (e.g., fever, abdominal tenderness, and a mass in the lower intestinal tract) and a serum specimen reactive to *A. costaricensis* antigen.

We solicited internists, clinical pathologists, and surgeons in hospitals in Guatemala City for case reports and asked pathologists to estimate the number of cases they had diagnosed in the past 5 years. For logistic reasons, only patients living in Guatemala City whose cases were diagnosed on or after 1 January 1995 and reported to us by 10 August 1995 were included in the investigation.

Retrospective cohort study. The company provided us with detailed menus for all eight business luncheons held in January and February 1995 and a list of the 37 persons who attended or who served at one or more luncheons. We asked the persons enrolled in the study what they had consumed at the luncheons and whether they had had a gastrointestinal illness during the period of January through March 1995. We also obtained information about other exposures (e.g., consumption of drinking water and various vegetables, fruits, herbs, fish, and shellfish at home or in a restaurant) during the 2-month period before the onset of illness for cases and during January and February for persons without gastrointestinal illness.

Case-control study. For each case enrolled in the study, we selected one to three neighbors or friends as controls who were matched by age (±5 years for adults 18 years of age or older and ±2 years for children), sex, socioeconomic status (occupation and educational level were considered), and the area of Guatemala City in which they lived. To be eligible, controls must not have been seroreactive to *A. costaricensis* antigen (see below), have been outside Guatemala City for >2 weeks, or have had a gastrointestinal illness during the 2-month period before the date of onset of the referent case’s symptoms and must have lived in Guatemala City for at least 1 year before the onset date. We interviewed cases and controls about their dietary habits and other exposures during the 2-month period before the referent case became ill. Of note, all cases but none of the well persons from the cohort study were included in the case-control study.

Histopathology and Clinical Laboratory Investigation

All tissue specimens were initially examined by pathologists in Guatemala. Slides of specimens from five cases were also reviewed by the Centers for Disease Control and Prevention (CDC). Serum specimens were tested with a latex agglutination test for reactivity to crude *A. costaricensis* antigen by the Department of Pathology, Hospital San Juan de Dios in San José, Costa Rica [27]. Specimens from all well persons in the cohort study and from the cases who provided specimens were tested once, whereas specimens from the 17 controls who provided specimens were tested in duplicate. Seroreactive specimens were tested by the CDC for antibody to *Trichinella spiralis* by EIA (Trichinella microtiter ELISA, catalog no. TN-1, LMD Laboratories, Carlsbad, CA [35a]) and by bentonite flocculation [36] as well as for antibody to *Toxocara canis* by EIA [37, 38]. Serum specimens from the possible cases who were known to have elevated transaminase levels were tested by the CDC for IgM antibody to hepatitis A virus (HAV), IgM antibody to hepatitis B core antigen, IgM antibody to hepatitis B surface antigen, and total antibody to hepatitis C virus (HCV) by using HAVAB-EIA, CORAB RIA, AUSRIA-II RIA, and HCV EIA, respectively (Abbott Laboratories, Chicago). In addition, these specimens were tested for total antibody to hepatitis E virus [39].

Food and Environmental Investigation

We obtained information about the preparation of implicated food items from persons enrolled in the case-control study and restaurant chefs and information about the origin of implicated food items from a convenience sample of six vendors at a local market in Guatemala City. We interviewed two mint farmers from farms ~20 miles west of Guatemala City about farming and harvesting practices, climatic conditions, the presence of slugs and rats in their fields, and pesticide use. We examined slugs and planarians that we collected on these farms for *A. costaricensis* larvae after digestion in artificial gastric juice (i.e., 5 g of pepsin and 7 mL of hydrochloric acid in 1 L of distilled water). We obtained monthly meteorologic data for Guatemala City for the period of January 1985 through July 1995 from the Instituto Nacional de Sismología, Vulcanología, Meteorología e Hidrología, Guatemala City.

Statistical Analysis

We used Epi-Info Version 6.04 [40] to calculate univariate 95% confidence intervals for relative risks by the Taylor series
approximation, two-tailed $P$ values by use of Fisher’s exact test, and maximum likelihood estimates for matched odds ratios, exact confidence intervals, and $P$ values. When the sample size was small or the estimate for an odds ratio was infinite, we used StatXact to calculate exact odd ratios, confidence intervals, and $P$ values [41]. Conditional logistic regression was used to attempt multivariate analysis of data from the matched case-control study.

Results

Annual Incidence of Abdominal Angiostrongyliasis in Guatemala

During the 5-year period of 1990 through 1994, the eight pathologists (from six different hospitals) we interviewed had evaluated an estimated total of two histopathologically confirmed cases of abdominal angiostrongyliasis per year and approximately eight cases of intestinal eosinophilia per year, some of which were consistent with abdominal angiostrongyliasis. In contrast, during the 7.5-month period of 1 January through 10 August 1995, seven of the eight pathologists diagnosed a total of 22 cases, including 15 histopathologically confirmed and seven probable or possible cases.

Cohort Study

From 17 May through 15 June 1995, we contacted 27 (73.0%) of the 37 persons who attended or served at any of the eight business luncheons and enrolled them in the cohort study. Among the 27 persons, we identified two confirmed cases and one probable case (attack rate, 11.1%). The three cases were men aged 32, 47, and 68 years, respectively, all of whom were hospitalized. The median age of the 24 well persons was 41 years (range, 22–57 years); 20 (83.3%) were male. All three cases had serum specimens reactive to $A. \text{costaricensis}$ antigen, and 23 well persons were seronegative; one well person was seroreactive and was excluded from further analysis. Only three of the eight luncheons were attended by all three cases; neither attendance at any of the eight luncheons nor consumption of a food item served at the three luncheons attended by all three cases was implicated by our analysis. However, consumption of raw shrimp at home or in a restaurant (73.0%) of the 37 persons who attended or served at any of the eight business luncheons and enrolled them in the cohort study was strongly associated with illness (table 1). Although persons who ate raw abalone (marine shellfish) ceviche (a traditional fish or shellfish dish, see below) were 11 times more likely to become ill, this association did not quite achieve statistical significance ($P = .052$).

<table>
<thead>
<tr>
<th>Raw food item*</th>
<th>Exposed</th>
<th>Not exposed</th>
<th>RR$^1$</th>
<th>$P$ value$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mint</td>
<td>2/7</td>
<td>1/18</td>
<td>5.1</td>
<td>.18</td>
</tr>
<tr>
<td>Ceviche$^a$</td>
<td>2/4</td>
<td>1/22</td>
<td>11.0</td>
<td>.052</td>
</tr>
<tr>
<td>Abalone</td>
<td>1/6</td>
<td>1/19</td>
<td>3.2</td>
<td>.43</td>
</tr>
<tr>
<td>Shrimp</td>
<td>2/16</td>
<td>0/9</td>
<td>Undefined</td>
<td>.52</td>
</tr>
<tr>
<td>Fish</td>
<td>0/1</td>
<td>3/24</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Caracol$^a$</td>
<td>2/3</td>
<td>1/22</td>
<td>14.7</td>
<td>.029</td>
</tr>
</tbody>
</table>

Table 1. Results of a cohort study of an outbreak of abdominal angiostrongyliasis: attack rates and relative risks by type of raw food consumption at home or in a restaurant.

* Selected items only; compare with results for case-control study (table 3).
† Persons who did not remember if they had eaten a particular item were excluded from the analysis for that variable.
‡ Univariate analysis.
§ Fisher’s exact test.
$^a$ Traditional fish or shellfish dish, which reportedly contained raw mint; see Results for details.
* Freshwater snail.

Figure 1. Results of a case-control study of an outbreak of abdominal angiostrongyliasis by month of symptom onset ($A$) and month of diagnosis ($B$) in Guatemala from December 1994 through August 1995 (18 cases). Includes only patients whose cases were reported from 1 January through 10 August 1995; see Methods for details.

Case-Control Study

Four cases (18.2% of 22) who had confirmed abdominal angiostrongyliasis were not enrolled in the case-control study because of refusal to participate (three) or residence outside of Guatemala City (one). Of the 18 cases enrolled in the study, including all three from the cohort study, 11 (61.1%) had confirmed infection, 3 (16.7%) had probable infection, and 4 (22.2%) had possible infection. The cases had a median age of 37 years (range, 9–68 years; only one was younger than 18 years of age), and 11 (61.1%) were male. We excluded four (10.8%) of the 37 possible controls we identified because they had a serum specimen reactive to $A. \text{costaricensis}$ antigen. The median age of the 33 controls we enrolled was 36 years (range, 9–63 years), and 21 (63.6%) were male. Thirteen (72.2%) of the 18 cases and 23 (69.7%) of the 33 controls had at least a postgraduate university degree or were senior executives. Cases and controls were interviewed from 14 May through 15 December 1995: cases a median of 91 days (range, 19–178 days) after the onset of symptoms (figure 1) and controls a median
In the referent case (13 (72.2%) underwent abdominal surgery. A 24-year-old case and weight loss (table 2); 15 (83.3%) were hospitalized, and
nal pain, fever, loss of appetite, diarrhea and/or constipation, E or chronic hepatitis B or C.
characteristics of the 11 confirmed cases and the seven probable American, sources; caracoles (§) and probable or possible cases were comparable. However, probable or possible cases were less likely to be hospitalized (P = .017) and to have undergone surgery (P < .001) than were confirmed cases.
Consumption of the following six raw food items was associated with illness: mint (“hierbabuena”; family, Labiatae; genius, Mentha), shrimp, and all four types of ceviche about which we asked in the questionnaire (all of which reportedly contained raw mint) (table 3). The results were comparable for confirmed and probable or possible cases and remained essentially unchanged when the three cases from the cohort study were excluded from the analysis. In the 2 months before the onset of illness, 17 (94.4%) of the 18 cases, all adults, had eaten at least one (median, three) of the six implicated raw foods, and all 17 had eaten at least one food item that contained raw mint. In contrast, 22 (66.7%) of the 33 controls had eaten at least one (median, two) of the six implicated raw food items, and 21 (63.6%) had eaten at least one food item that contained raw mint (OR for consumption of mint or ceviche that contained mint, 7.0; 95% CI, 1.0–315). Of note, all 18 cases and 29 (87.9%) of the 33 controls had eaten raw lettuce (OR, undefined).

**Histopathology and Clinical Laboratory Investigation**

In the case-control study, tissue specimens were available for review from 14 (77.8%) of the 18 cases (11 confirmed cases and three probable or possible cases). All specimens had intense eosinophilic infiltration of the intestinal wall that was associated with granulomatous reaction and eosinophilic vasculitis. In eight (57.1%) of the 14 specimens, A. costaricensis worms (figures 2 and 3) or larvae (figure 4) were noted in the mesenteric arteries or intestinal wall. In three specimens (21.4%), eggs compatible with A. costaricensis were noted in small arterioles or the intestinal wall (figure 5). No worms, eggs, or larvae were found in three specimens (21.4%).

Serum specimens from 15 (83.3%) of the 18 cases (eight confirmed cases and seven probable or possible cases) were tested for reactivity to A. costaricensis antigen. Thirteen (86.7%) of the 15 specimens were reactive, and two (13.3%; both from confirmed cases) were negative. Three (23.1%; all from confirmed cases) of the 13 seroreactive specimens were also reactive to T. canis antigen. All 15 specimens were negative for antibody to T. spiralis. Two possible cases, as well as one confirmed case and one probable case, had elevated transaminase levels (table 2); neither possible case who was tested had serological evidence of acute hepatitis A, B, C, or E or chronic hepatitis B or C.

**Food and Environmental Investigation**

**Fish and ceviche.** In Guatemala City, fish, shrimp, and abalone come from both domestic and foreign, mostly Central American, sources; caracoles (Pomacea ghiesbreghii Reeve,
Table 3. Results of case-control study of an outbreak of abdominal angiostrongyliasis: raw food items implicated.

<table>
<thead>
<tr>
<th>Raw food item*</th>
<th>Cases (n = 18)</th>
<th>Controls (n = 33)</th>
<th>OR (95% CI)²</th>
<th>P value³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mint</td>
<td>14/18</td>
<td>11/33</td>
<td>6.9 (1.5–66.0)</td>
<td>.009</td>
</tr>
<tr>
<td>Ceviche</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abalone</td>
<td>9/17</td>
<td>4/33</td>
<td>10.9 (1.4–492)</td>
<td>.011</td>
</tr>
<tr>
<td>Shrimp</td>
<td>11/16</td>
<td>10/33</td>
<td>8.5 (1.1–394)</td>
<td>.032</td>
</tr>
<tr>
<td>Fish</td>
<td>12/17</td>
<td>13/33</td>
<td>8.5 (1.1–394)</td>
<td>.032</td>
</tr>
<tr>
<td>Caracol</td>
<td>7/18</td>
<td>2/33</td>
<td>5.3 (1.0–53.3)</td>
<td>.034</td>
</tr>
<tr>
<td>Any dish with raw mint*</td>
<td>17/18</td>
<td>21/33</td>
<td>7.0 (1.0–315)</td>
<td>.029</td>
</tr>
<tr>
<td>Shrimp</td>
<td>7/18</td>
<td>4/33</td>
<td>Infinite (1.4 to infinite)</td>
<td>.049</td>
</tr>
</tbody>
</table>

NOTE. Analysis includes three cases who also were included in the cohort study.
* Compare with results from cohort study (table 1).
¹ Persons who did not remember if they had eaten a particular item were excluded from the analysis for that variable.
² Exact OR (see Methods for details), 95% CI, and P values for univariate matched analysis.
³ Traditional fish or shellfish dish, which reportedly contained raw mint; see Results for details.
‡ Freshwater snail.
§ Mint or any of the four types of ceviche listed.

1856) are collected in local streams. Ceviche consists of one or more kinds of raw or cooked fish or shellfish marinated in lemon juice and mixed with one or more raw herbs shortly before serving. Mint was the only herbal ingredient added by all persons who were interviewed about food preparation (i.e., nine persons from the case-control study and five restaurant chefs). Two other raw herbs, coriander (cilantro) and parsley, were each added by only one-third of the interviewees.

Mint farms. Mint is grown on densely planted patches, ~1 m wide and 10–15 m long. The two farms we visited had 25 or 50 such patches; an entire patch is harvested when the plants are ~30 cm high. After mint is harvested, it is rinsed with water, stored in the farmhouse, and transported to market the next day. The mint plants regrow from the stumps for years, and the patches are harvested several times per year. Slugs are often found on the mint plants, and rats are present on the farms. In recent years, no increase in the density of slugs or rats had been noted, and farming practices had not changed. We did not find any A. costaricensis larvae in the 68 slugs (Limax marginatus Müller, 1774) and two planarians we collected from mint plants.

Rainfall. The monthly rainfall in Guatemala City for each month of the period of January 1994 through July 1995 was

Figure 2. Low-power photomicrograph of a section of terminal ileum from a patient with abdominal angiostrongyliasis. The well-demarcated nodule in the submucosa contains two sections of an adult Angiostrongylus costaricensis worm; the intense granulomatous reaction has obliterated any evidence that the worm is within a blood vessel (hematoxylin-eosin stain; scale bar = 200 μm).

Figure 3. High-power photomicrograph of the adult Angiostrongylus costaricensis worm shown in figure 2. The worm is markedly degenerated and has lost most of its morphological features; the multinucleate gut is still present (hematoxylin-eosin stain; scale bar = 10 μm).
Our conclusion that raw mint is the likely vehicle of infection for the outbreak is based on epidemiological evidence. The association with several ceviche dishes that we found in univariate analysis can be explained by the fact that the dishes reportedly included raw mint. However, we cannot rule out the possibility that freshwater snails and other shellfish may also become infected with *A. costaricensis* and serve as sources for infective-stage larvae, as has been described for *A. cantonensis* [21]. To assess the independent contributions of the implicated raw mint, ceviche, and shrimp dishes, which were highly correlated exposure variables, we attempted matched logistic regression with use of forward variable selection; however, the analysis was limited by the small sample size, and the results were inconclusive.

Our investigation had several other limitations. First, because the incubation period for abdominal angiostrongyliasis in humans is unknown, our questionnaire may not have covered the relevant period of exposure for all cases. Second, although our interview data indicated that raw mint was an ingredient of ceviche in Guatemala City, we could not assess whether all ceviche dishes eaten by cases contained mint. Third, cases may not have recalled their exposures accurately, especially if interviewed several months after the onset of illness [42]. Controls, who, on average, were interviewed >9 weeks after their referent cases may have recalled exposures even less accurately. However, the food-exposure data we obtained are in essence food-preference data (i.e., data about whether a person is likely to have eaten something); such data may be more accurate for the food items implicated in this investigation (e.g., various types of ceviche) than for less notable items.

Fourth, in our questionnaire, we may have omitted questions about relevant, but to date unidentified, risk factors for abdominal angiostrongyliasis. Fifth, the sensitivity (in our study only 86.7%) and specificity of the serological test that uses crude *A. costaricensis* antigen has not been fully evaluated. However,
none of these limitations is a likely explanation for the strong association we found between consumption of mint and illness.

The mode of contamination of the mint remains unclear. Previous studies have shown a low prevalence of infection with *A. costaricensis* in slugs (zero to 1.3% [43]) and rats (2.3% [44]) in Guatemala. This finding suggests either that the mint leaves were contaminated with the mucous secretions of infected mollusks [20] or that infected mollusks or planarians remained adherent to the mint leaves when they were washed. Although we did not find *A. costaricensis* larvae in the slugs and planarians we examined, they were collected >1 year after the outbreak occurred and from only two mint farms.

Because human abdominal angiostrongyliasis can be a serious illness and no regimen of antiparasitic treatment has been identified, prevention is key. All potentially contaminated foods should be cooked; vegetables and fruits that are eaten raw should be cleaned thoroughly to remove mollusks, planarians, and possible larval contamination. These recommendations were used in a health education campaign in the South Pacific islands that was associated with a marked decrease in the number of cases of food-borne eosinophilic meningitis [21]. Only limited experimental data are available about the inactivating of *A. costaricensis* larvae by disinfectants [45], and no data are available about their practical use and their ability to inactivate larvae within mucus secretions, mollusks, or planarians that adhere to produce. Although we do not yet know whether freshwater snails, shellfish, and other animals are ever infected with *A. costaricensis*, adequate cooking of snails and shellfish would prevent infection; marinating in lemon juice (e.g., in ceviche) might not kill the larvae.

Our investigation suggests that *A. costaricensis* may be food borne and cause outbreaks, and it underscores the need for a better understanding of the risk factors for and prevalence of human abdominal angiostrongyliasis. To improve detection of cases and outbreaks, the possibility of a food-borne outbreak should be considered when cases of abdominal angiostrongyliasis are diagnosed.

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