An outbreak of hepatitis A associated with consumption of raw blueberries.

L Calder *
G Simmons *
C Thornley †
P Taylor *
K Pritchard *
G Greening †
J Bishop ‡

Address for correspondence and reprints:
Lester Calder
Public Health
Community Services
Auckland District Health Board
Private Bag 92 605
Symonds St
AUCKLAND 1
Telephone: 64 9 623 4620
Fax: 64 9 630 7431
E-mail: lesterc@adhb.govt.nz.

Word count: Text including tables, excluding title page, figure and references 2959
References 383

Running head: Blueberries and hepatitis A

* Public Health, Community Services, Auckland District Health Board
† Kenepuru Science Centre, Institute of Environmental Science & Research Limited
‡ Ministry of Health
Summary

The aim of this report is to describe the epidemiology, investigation and control of a hepatitis A (HAV) outbreak in New Zealand. Descriptive and analytical epidemiology, virology, product traceback and an orchard investigation were carried out. A case-control study involving 39 cases revealed that 56% of cases had consumed raw blueberries, as compared with 14% of controls (odds ratio 7.6; 95% confidence intervals 2.6 – 22.4). Traceback of product through retailers and wholesalers implicated a single commercial orchard. DNA sequencing showed more than 90% similarity between HAV obtained from cases’ faeces and that found in product from the orchard. Sanitary audit of the orchard revealed multiple opportunities for contamination of product by pickers. This outbreak highlights the need for food safety programmes in the berry fruit industry.
Introduction

The incidence of notified hepatitis A in New Zealand has declined in recent decades, from a rate of 145.7 per 100,000 population in 1971 \(^1\) to 1.6 in 2001 \(^2\). Overseas travel is currently the most common risk factor reported by notified cases, followed by consumption of known or potentially contaminated food or water \(^2\).

In the first three months of 2002 in Auckland, New Zealand, there was a sharp increase in the number of cases of hepatitis A notified to the public health office. An increase was also reported from other health districts in New Zealand. The aim of this report is to describe the epidemiology, investigation and control of this outbreak.

Methods

Epidemiologic investigation

A case was defined as someone notified to a public health office in New Zealand with an acute illness with the following features: symptoms (including fever, malaise, anorexia, nausea or abdominal discomfort; jaundice or raised serum aminotransferase levels); and serum IgM antibodies against HAV (anti-HAV IgM). Unnotified cases were identified by obtaining positive anti-HAV IgM results from hospital and community laboratories. Cases reported between 1 January and 31 May 2002 were described in terms of time, place and person characteristics, and common risk factors for hepatitis A infection.

A hypothesis that blueberries were the source of illness was tested by a case-control study. Cases reported between 1 January and 10 April 2002 were eligible for enrollment in the study.
Controls were obtained for each case by telephoning sequentially the phone numbers on a randomly selected page of the telephone directory for the district in which their case resided. A new page was used for obtaining each control. It was intended to obtain two controls for each case. Potential controls were excluded if they were under 16 years of age; had had hepatitis of any kind (because it was impractical to exclude only those with confirmed previous hepatitis A); had received hepatitis A vaccination; had received an injection of immunoglobulin in the past six months; did not speak English; or were unable to answer questions (e.g. due to dementia, etc.).

Cases and controls were interviewed by telephone using a standardised questionnaire. The questionnaire included demographic information, questions on symptoms and hospitalisation (for cases only), and exposures during the case’s incubation period: foods consumed (berry fruits; shellfish; commercially prepared sandwiches, bakery products, salads and cold meats); recent contact with known hepatitis A; contact with children under five years of age; drinking water; travel and (for men only) sexual contact with other men. Interviewees, but not interviewers, were blinded to the study hypothesis. The incubation period was defined as two to seven weeks before the onset of symptoms in the case. Controls were asked about their exposures to potential risk factors based on their corresponding case’s onset date.

**Traceback investigation**

If the case had eaten blueberries and could remember the retailer and approximate date of purchase, information was obtained from the retailer on wholesale sources for the blueberries stocked. Wholesalers were then questioned about the orchards from which they had obtained their product.
**Site investigation**

One particular orchard was identified as a probable source (see *Results*). The orchard was investigated by a sanitary audit (toilets and hand hygiene); an inquiry into the quality of all water which may have contaminated the product; and a food safety audit based on hazard analysis critical control points (HACCP) 3.

**Data entry and statistical analyses**

Data from the case-control study were analysed using Epi Info version 6.04b4. Demographic characteristics of cases and controls were compared using the Kruskall-Wallis test. Univariate unmatched odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for exposures. Differences between case and control populations were tested using the chi-square method, with p values subject to Yates’ correction and Fisher’s exact test applied when expected values in any cell were less than five. Stratified analysis using the Mantel Haenszel method was performed to control for confounding between various exposures. All exposures with an OR exceeding 1 on univariate analysis were subjected to multivariate analysis to control for confounding between exposures. Stepwise conditional logistic regression analyses were performed, using SAS software5 to identify the combination of variables that best explained the differences between cases and controls.

**Virological investigation**

Faecal and blood or serum samples from cases and samples of stored blueberries from the orchard were analysed for the presence of HAV. Faecal suspensions were prepared as previously described 6. Acid flocculation was used to recover HAV from 100g of whole frozen blueberries 7. Virus was eluted from the surface of the fruit, then acidified and precipitated by centrifugation. The virus-containing pellet was resuspended in PBS and stored at -70°C.
Molecular analysis

Viral RNA was extracted from faecal suspensions and blueberry eluates, then transcribed to cDNA as previously described. Viral cDNA was amplified using conserved HAV primers HAV-CL and HAV-CR derived from the VP3 capsid gene of strain HM 175. PCR products were analysed by gel electrophoresis. Confirmation of HAV presence was carried out by dot blot hybridization as previously described, and using a biotinylated oligonucleotide probe, HAV-C3. The HAV reference strain, HM-175 was used as a positive control and RNase-free water as a negative control. Anti-contamination procedures were followed for all molecular procedures. Further characterisation was carried out by genetic sequencing. Blueberry and faecal HAV sequences were compared with reference HAV sequences deposited in the US National Institutes of Health NCBI GenBank database. Paired sequence analysis of HAV sequences from faecal and blueberry specimens was carried out using the BLAST programme.

Results

Descriptive epidemiology

There were 81 laboratory-confirmed cases of hepatitis A reported throughout New Zealand between 1 January 2002 and 31 May 2002 (figure 1).

Cases were distributed across 13 health districts, with the majority (60%) occurring in the three Auckland health districts. Eighteen (22.2%) were hospitalised and one case died. There was no apparent clustering by age (median = 23 years, range 0 to 88 years), gender (46.9% were male) or locality. When compared to cases notified in January to May 2000-1, there was no significant difference in ethnic distribution or in the proportion with a history of recent overseas travel. However a higher proportion of Auckland cases than usual were of European ethnicity (61.2% vs.
an average of 35.5% for 2000-01, chi-square=7.87, p=0.005) and people who had not travelled outside New Zealand (72.5% vs. an average of 52.9% for 2000-01, chi-square=4.06, p=0.04).

Interview of cases revealed that an unusual number came from higher socio-economic areas. This suggested a seasonal food source that was more expensive and not widely consumed. Inquiry about food exposures revealed no dining premises common to a number of cases but suggested consumption of blueberries as a common risk factor.

Analytical epidemiology

Of the 43 total eligible cases reported between 1 January and 10 April 2002, 39 were enrolled in the case-control study (participation rate 90.7%). Four eligible cases could not be contacted. The participation rate among 98 eligible controls was 78%. The characteristics of cases and controls are shown in Table 1. They did not differ significantly by age, sex, or ethnicity.

Among the 39 cases, 29 (74.4%) reported nausea, 19 (48.7%) vomiting, 12 (30.8%) diarrhoea, 26 (66.7%) fever, 21 (53.8%) muscle aches, 27 (69.2%) headache, 25 (64.1%) abdominal pains, 35 (89.7%) tiredness, 31 (79.5%) jaundice, 38 (97.4%) dark urine, and 21 (53.8%) pale stools. The median duration of symptoms (for 35 cases with a known date of cessation of symptoms) was 21 days, range 5-165 days.

During the two to seven week period before the onset of illness, 56% of cases had consumed raw blueberries, as compared with 14% of controls (OR 7.6; 95% CI 2.6 – 22.4) (Table 2).

Consumption of raw or cooked blueberries was also significantly associated with illness (OR 2.9; 95% CI 1.2 – 7.5), although after stratification by consumption of raw blueberries the resulting Mantel Haenszel odds ratio was not significantly different from 1.0 (Mantel Haenszel OR 0.26, 95% CI 0.03 – 2.15). No other exposures were significantly associated with illness. Logistic
regression analysis showed that only consumption of raw blueberries had an independent statistically significant association with disease (adjusted OR 8.29, 95% CI 3.09 - 22.24; population attributable risk 51.01%, 95% CI 49.01% - 53.02%).

**Blueberry traceback investigation**

A trace back of product consumed by hepatitis A cases notified in Auckland between 7 February and 30 April 2002 revealed that, of 17 cases who had eaten fresh blueberries, 14 consumed blueberries from one source orchard. It was not possible by traceback to link any one case exclusively to blueberries from the orchard as all of the stores also received stock from other orchards. Fourteen tonnes of blueberries from the orchard had been sold in New Zealand, 14 tonnes had been exported and 22 tonnes were in cold storage.

**Site investigation**

The orchard maintained no records of illness absences. None of the sixty orchard workers reported symptoms of hepatitis A, though not all workers were questioned and no serological testing was carried out. A nine year old child who had been present at the orchard during the harvest in late December and early January developed symptoms compatible with hepatitis A and was IgM-positive on 29 January 2002. It was reported that the child had not handled the product.

Workers did not wear gloves when handling product and a food safety audit revealed multiple opportunities for an infected worker to faecally contaminate the product or processing equipment during picking and packing.
The only toilet facilities available to workers in the fields were pit latrines without running water or soap. There was no system for removal of rubbish such as disposable nappies left by the pickers. The packing shed had a flush toilet with running water, soap and towel.

One of the three pit latrines on one of two blocks constituting the orchard were located in the middle of the blueberry plants. The other two latrines were 30 metres from blueberry plants. During the site inspection the effluent level in the latrines was four feet below the ground surface. High rainfall during the harvest season may have raised the ground water level. Rainfall in the district during December 2001 was 203.4mm. This was 2.8 times the average for the same month over the preceding four years (Data supplied by the National Institute of Water and Atmospheric Research, 13 June 2002).

Drinking water at the orchard was obtained from town supply and stored in a concrete tank. The drinking water and groundwater were not tested for HAV or faecal coliforms. The orchard was not irrigated by bore or stream water. Human or animal faeces were not used to fertilise the orchard.

**Virological investigation**

**Biological samples**

HAV was successfully detected in 5/9 stool samples from cases, but not detected in blood or serum samples. The median interval from onset of symptoms to stool specimen collection was 12 days (range 8-19 days) for the five cases with positive samples.
Food samples

HAV was detected by RT-PCR in 3/6 samples of stored frozen blueberries from the coolstore.

DNA sequencing of PCR products from 1/3 HAV-positive blueberry samples and 3/5 HAV-positive faecal specimens confirmed the presence of HAV. Sequence analysis of 170bp fragments showed more than 90% similarity between the sequences from faecal and blueberry specimens, the HAV reference strain HM-175 and HAV sequences deposited in the NCBI Genbank database.

Control measures

On 12 April 2002 all unsold product harvested from the orchard between 23 December 2001 and the end of January 2002 was impounded. This product remains in frozen storage and will only be released after processing into a form which would ensure inactivation of HAV.

On 12 April, based on the descriptive epidemiology and the case-control study implicating blueberries, the Director General of Health, issued a legally privileged statement via the national news media warning the public not to consume blueberries raw if they were purchased between 23 December and the end of January. This course of action was taken before hepatitis A contamination of the blueberries was virologically confirmed.

On 30 May, when trace-back was complete, a public health alert was sent to four countries to which product from the orchard had been exported along with a request to notify the New Zealand Ministry of Health of any cases thought to be linked to the exported blueberries. No cases were notified by these countries.
HACCP-based food safety programmes covering berry fruit production and processing are being developed by Blueberries New Zealand and Vegfed. In response to the outbreak some wholesalers of blueberries have adopted an approved supplier policy whereby only product from producers with approved food safety programmes will be marketed.

**Discussion**

We have described a multi-district New Zealand outbreak of hepatitis A associated with the consumption of contaminated blueberries. We were unable to define the mode of contamination of the product but likely causes include contamination at the orchard by infected food handlers or by faecally polluted groundwater. We were unable to determine the role of the child case who attended the orchard. He may (along with other pickers) have been the source for the outbreak, or he may have himself been infected by blueberries which had been contaminated by pickers or by groundwater.

Outbreaks of hepatitis A have been associated with consumption of lettuce\(^9\), frozen strawberries\(^{10, 11}\) and frozen raspberries\(^{12, 13}\). The multi-state outbreak associated with strawberries\(^{10}\) involved over 250 cases. USFDA investigators traced the source of the product to Mexico and found that the strawberry fields had open-pit latrines, and workers had no ready way to wash their hands\(^{14}\).

Despite nation-wide distribution of 14 tonnes of product from the orchard, only 20 cases were identified who reported blueberry consumption. This suggests that contamination was at a low level and/or not uniform. A similar phenomenon has been noted in other outbreaks\(^{10, 11}\). The high contamination rate we found among blueberries (3/6 samples) may reflect the fact that our sampling procedure was not random.
The elevated OR of 7.6 for blueberry consumption is unlikely to be due to chance, bias or confounding. Nonetheless there are several potential sources of bias in this study.

Our recruitment of controls from telephone directories was potentially biased by the fact that although 92% of New Zealand households have telephones\textsuperscript{15}, 15% of them are unlisted in public directories (personal communication Karen Witten, Alcohol and Public Health Research Unit). Cases and controls differed in their ethnic distribution.

Recall of dietary history over four to eight weeks is of questionable accuracy. Since cases and controls were questioned about the same exposure period this bias is likely to have been similar for both groups. Blueberries are a relatively expensive and infrequently-consumed fruit in New Zealand, which may have improved the accuracy of recall. The study was stopped after the Ministry of Health’s public warning had revealed the study hypothesis.

This outbreak highlights the need for HACCP-based food safety programmes\textsuperscript{3} in the berry fruit industry. It has been demonstrated that handwashing with adequate volumes of water or with antibacterial soap or ethanol reduces experimental HAV contamination during handling of lettuce \textsuperscript{16}. Hand hygiene and illness exclusion policies are essential in the berry fruit industry.
Acknowledgements

We gratefully acknowledge the assistance of public health staff in Auckland, Waikato, Northland Rotorua, Wellington/Hutt and Southland health districts in conducting interviews; the cooperation of the orchard owner; the contribution of the Ministry of Health and Wellington and Waikato Public Health offices in product tracing; K Gilmore of Kenepuru Science Centre, Institute of Environmental Science & Research Limited for statistical analysis; and the following for assistance with hepatitis serology results: Dr Arthur Morris at Diagnostic Medlab; Dr Margaret Croxson at LabPlus, Auckland District Health Board; Dr Susan Taylor, Counties-Manukau District Health Board and Ms Janet Wilson, Southern Community Laboratories.
References


Table 1. Demographic characteristics of cases and controls

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Cases (n=39)</th>
<th>Controls (n=71)</th>
<th>Test statistic (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range)</td>
<td>40 (16 – 88)</td>
<td>48 (15 – 88)</td>
<td><strong>Kruskall-Wallis</strong> H = 3.50 (0.06)</td>
</tr>
<tr>
<td>16-24</td>
<td>11</td>
<td>10</td>
<td>$\chi^2 = 4.35 (0.23)$</td>
</tr>
<tr>
<td>25-44</td>
<td>12</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>45-64</td>
<td>12</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>≥ 65</td>
<td>4</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>(\chi^2 = 0.56 (0.45))</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td>(\chi^2 = 8.35 (0.051))</td>
</tr>
<tr>
<td>European</td>
<td>29</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Maori</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Pacific Island</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Not recorded</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Frequency of selected exposures among cases and controls

<table>
<thead>
<tr>
<th>Exposure</th>
<th>No. cases exposed / total responding§ (%)</th>
<th>No. controls exposed / total responding (%)</th>
<th>Univariate odds ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberries (raw or cooked)</td>
<td>20/34 (58.8)</td>
<td>23/70 (32.9)</td>
<td>2.92 (1.15 – 7.51)</td>
<td>0.012</td>
</tr>
<tr>
<td>Blueberries (raw)</td>
<td>19/34 (55.9)</td>
<td>10/70 (14.3)</td>
<td>7.60 (2.64 – 22.41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Raspberries (raw or cooked)</td>
<td>12/34 (35.2)</td>
<td>15/71 (21.1)</td>
<td>2.04 (0.74 – 5.59)</td>
<td>0.12</td>
</tr>
<tr>
<td>Raspberries (raw)</td>
<td>12/34 (35.3)</td>
<td>13/70 (18.6)</td>
<td>2.39 (0.85 – 6.74)</td>
<td>0.06</td>
</tr>
<tr>
<td>Apricots</td>
<td>3/32 (9.4)</td>
<td>6/70 (8.6)</td>
<td>1.10 (0.17 – 5.60)</td>
<td>0.89</td>
</tr>
<tr>
<td>Melon</td>
<td>5/32 (15.6)</td>
<td>9/70 (12.9)</td>
<td>1.26 (0.30 – 4.64)</td>
<td>0.71</td>
</tr>
<tr>
<td>Kiwifruit</td>
<td>3/32 (9.4)</td>
<td>4/70 (5.7)</td>
<td>1.71 (0.23 - 10.74)</td>
<td>0.50</td>
</tr>
<tr>
<td>Watermelon</td>
<td>2/32 (6.3)</td>
<td>3/70 (4.3)</td>
<td>1.49 (0.12 - 13.65)</td>
<td>0.67</td>
</tr>
<tr>
<td>Mandarins</td>
<td>2/32 (6.3)</td>
<td>3/70 (4.3)</td>
<td>1.49 (0.12 - 13.65)</td>
<td>0.67</td>
</tr>
<tr>
<td>Grapes</td>
<td>5/32 (15.6)</td>
<td>9/70 (12.9)</td>
<td>1.26 (0.30 - 4.64)</td>
<td>0.71</td>
</tr>
<tr>
<td>Shellfish (raw or cooked)</td>
<td>16/36 (44.4)</td>
<td>29/70 (41.4)</td>
<td>1.13 (0.46 – 2.75)</td>
<td>0.77</td>
</tr>
<tr>
<td>Shellfish (raw)</td>
<td>10/34 (29.4)</td>
<td>9/66 (13.6)</td>
<td>2.64 (0.84 – 8.32)</td>
<td>0.06</td>
</tr>
<tr>
<td>Commercial vegetable salad, raw or cooked</td>
<td>16/36 (44.4)</td>
<td>24/70 (34.3)</td>
<td>1.53 (0.62 – 3.78)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

§ Denominator excludes participants who answered ‘don’t know’ to question
<table>
<thead>
<tr>
<th>Scenario</th>
<th>n/100 (Proportion)</th>
<th>n/100 (Expected)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial vegetable salad, raw</td>
<td>14/34 (41.2)</td>
<td>20/66 (30.3)</td>
<td>1.61 (0.62 – 4.20)</td>
<td>0.28</td>
</tr>
<tr>
<td>Commercially prepared bakery item</td>
<td>19/35 (54.3)</td>
<td>28/69 (40.6)</td>
<td>1.74 (0.70 – 4.33)</td>
<td>0.19</td>
</tr>
<tr>
<td>Ready-to-eat meat product</td>
<td>27/35 (77.1)</td>
<td>54/71 (76.1)</td>
<td>1.06 (0.37 – 3.12)</td>
<td>0.90</td>
</tr>
<tr>
<td>Non-town supply water</td>
<td>13/37 (35.1)</td>
<td>22/71 (31.0)</td>
<td>1.21 (0.47 – 3.06)</td>
<td>0.66</td>
</tr>
<tr>
<td>Overseas travel during incubation period in case</td>
<td>8/39 (20.5)</td>
<td>8/71 (11.3)</td>
<td>2.03 (0.61 – 6.76)</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Figure 1. Epidemic curve for hepatitis A outbreak 2002, by date of reporting and blueberry consumption, also showing average weekly reports of hepatitis A for January to May 2000-1
Week of disease report

- Fresh blueberry eaters
- Non-blueberry eaters
- 2000-01 average

Public warning April 12 2002

Reported cases

d:\data\cook\desktop\produce docs\hepa in raw blueberries 2002.doc  09/03/2007