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An outbreak of hepatitis A associated with consumption of raw blueberries

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SUMMARY

This report describes 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 revealed that 56% of 39 cases had consumed raw blueberries, compared with 14% of 71 controls (odds ratio 7·6; 95% confidence intervals 2·6–22·4). Traceback of product through retailers and wholesalers implicated a single commercial orchard. Hepatitis A virus was detected by reverse transcriptase polymerase chain reaction in faecal specimens from cases as well as a blueberry product from the orchard. Presence of hepatitis A virus was confirmed by DNA hybridization and sequencing of PCR products. Sanitary audit of the orchard revealed multiple opportunities for contamination of blueberries by pickers. This outbreak highlights the need for food safety programmes in the berry fruit industry.

INTRODUCTION

The incidence of notified hepatitis A (HAV) 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 3 months of 2002 in Auckland, the number of cases of hepatitis A notified to the public health office increased sharply. An increase was also reported from other health districts in New Zealand. This paper describes the epidemiology, investigation and control of this outbreak.

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METHODS

Epidemiological investigation

A case was defined as a person notified to a public health office in New Zealand who met all of the following three criteria:

(1) one or more of the following symptoms: fever, malaise, anorexia, nausea or abdominal discomfort;
(2) jaundice or raised serum aminotransferase levels;
(3) serum IgM antibodies against HAV (anti-HAV IgM).

Other cases were identified from positive anti-HAV IgM results from hospital and community laboratories. Notifications were then obtained from these cases' doctors and they were included in the case control study (see below). Cases were described in

terms of time, place and person characteristics, and common risk factors for hepatitis A infection. Eligible exposed contacts were offered human normal immunoglobulin prophylaxis.

A hypothesis that blueberries were the source of illness was tested by a case–control study. Cases reported between 1 January and 12 April 2002 were eligible for enrolment in the study. Cases after 12 April were excluded because on that date the Director-General of Health's statement publicized the risk associated with blueberry consumption which meant that cases could no longer be blinded to the study hypothesis. Cases whose interviews had led to the formulation of the blueberry hypothesis were included in the study but were not informed of the study hypothesis.

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 6 months; did not speak English; or were unable to answer questions (e.g. due to dementia).

Cases and controls were interviewed by telephone using a standardized questionnaire. The questionnaire included demographic information, questions on symptoms and hospitalization (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 5 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 2–7 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 6.04d [4]. 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 Kruskal–Wallis test for continuous variables and the χ^2 method for categorical variables, with *P*-values subject to Yates' correction and Fisher's exact test applied when expected values in any cell were less than 5. 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 software [5] 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 100 g 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 $-70\ ^{\circ}C$.

Molecular analysis

Viral RNA was extracted from faecal suspensions and blueberry eluates, then transcribed to cDNA as previously described [6]. Viral cDNA was amplified

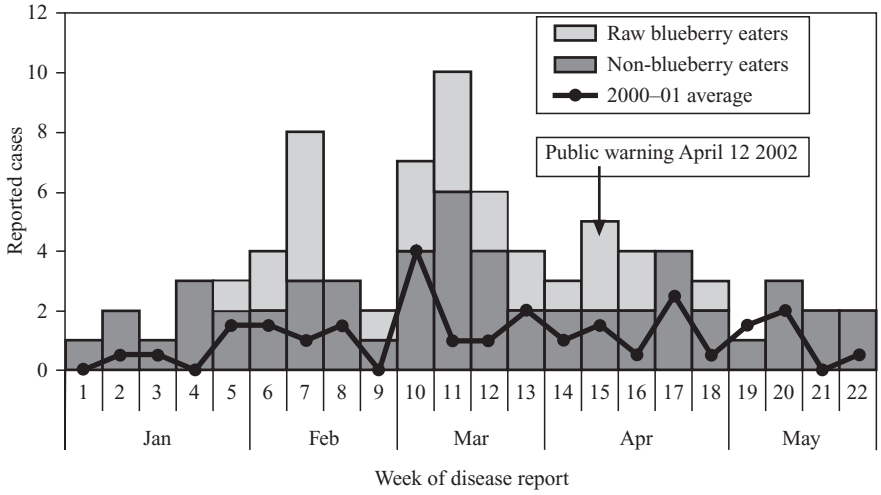

Fig. 1. Epidemic curve for hepatitis A outbreak 2002, by date of reporting and blueberry consumption, also showing average weekly reports of hepatitis A for January to May in 2000 and 2001.

using conserved HAV primers HAV-CL and HAV-CR derived from the VP3 capsid gene of strain HM 175 [8]. PCR products were analysed by gel electrophoresis. Confirmation of HAV presence was carried out by dot–blot hybridization as previously described [6], and using a biotinylated oligonucleotide probe, HAV-C3 [8]. 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 confirmation of identity was carried out by DNA sequencing. PCR products were sequenced in both directions using Big Dye-terminator cycle sequencing methodology (Applied Biosystems, CA, USA) on an ABI 3100 Genetic Analyser. Sequences from blueberry and faecal specimens were compared with HAV sequences deposited in the US National Institutes of Health NCBI GenBank® database using the BLAST nucleotide pairwise similarity 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 (Fig. 1).

Cases were distributed across 13 health districts, with most (60%) in the three Auckland health districts. Eighteen (22·2%) patients were hospitalized and one died. Cases were not concentrated in any particular age group (median = 23 years, range 4–88 years), gender (47% were male) or locality. A higher proportion of Auckland cases than usual were of European ethnicity (61·2% compared to 35·5% for 2000 and 2001, $\chi^2 = 7{\cdot}87$, $P = 0{\cdot}005$) and had not travelled outside New Zealand (72·5% compared to 52·9% for 2000 and 2001, $\chi^2 = 4{\cdot}06$, $P = 0{\cdot}04$). No secondary cases were discovered. An unusually high 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 91%). Four eligible patients could not be contacted. The participation rate in 98 eligible controls was 78%. The characteristics of cases and controls are shown in Table 1. They did not differ significantly by age or gender. They differed in their ethnic distribution and this difference nearly attained statistical significance. No potential controls were excluded because they did not speak English.

Of the 39 cases, 29 (74%) reported nausea, 19 (49%) vomiting, 12 (31%) diarrhoea, 26 (67%) fever, 21 (54%) muscle aches, 27 (69%) headache, 25 (64%) abdominal pains, 35 (90%) tiredness, 31 (80%) jaundice, 38 (97%) dark urine, and 21 (54%) 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.

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Table 1. *Demographic characteristics of cases and controls*

	Cases ($n=39$)		Controls ($n=71$)		Test statistic (P-value)
	No.	%	No.	%	
Age (years)					
Median (range) …	40 (16–88)		48 (15–88)		Kruskal–Wallis $H=3{\cdot}50$ (0·06)
16–24	11	28·2	10	14·1	$\chi^2=4{\cdot}35$ (0·23)
25–44	12	30·8	19	26·8	
45–64	12	30·8	31	43·7	
⩾65	4	10·3	11	15·5	
Sex					
Male	16	41·0	24	33·8	$\chi^2=0{\cdot}56$ (0·45)
Female	23	59·0	47	66·2	
Ethnicity					
European	29	74·4	59	83·1	$\chi^2=8{\cdot}35$ (0·051)
Maori	2	5·1	6	8·5	
Pacific Island	4	10·3	0	0·0	
Other	2	5·1	6	8·5	
Not recorded	2	5·1	0	0·0	

During the 2–7 week period before the onset of illness, 19 (56%) cases had consumed raw blueberries, compared with 10 (14%) 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 raw blueberries and could remember where they had bought them, 14 consumed those 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 60 orchard workers reported symptoms of hepatitis A, though not all workers were questioned and no serological testing was carried out. A 9-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 contaminate the product or processing equipment faecally during picking and packing.

The only toilet facilities available to workers in the fields were pit latrines without running water, soap or towels. 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.

The orchard had three pit latrines. One was in the middle of blueberry plants; the other two were 30 m from the plants. During the site inspection the effluent level in the latrines was 4 ft 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 203·4 mm. This was

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Table 2. *Frequency of selected exposures among cases and controls*

Exposure	No. cases exposed/ total responding* (%)	No. controls exposed/ total responding* (%)	Univariate odds ratio (95% CI)	*P*
Blueberries (raw or cooked)	20/34 (58·8)	23/70 (32·9)	2·92 (1·15–7·51)	0·012
Blueberries (raw)	19/34 (55·9)	10/70 (14·3)	7·60 (2·64–22·41)	<0·001
Raspberries (raw or cooked)	12/34 (35·2)	15/71 (21·1)	2·04 (0·74–5·59)	0·12
Raspberries (raw)	12/34 (35·3)	13/70 (18·6)	2·39 (0·85–6·74)	0·06
Apricots	3/32 (9·4)	6/70 (8·6)	1·10 (0·17–5·60)	0·89
Melon	5/32 (15·6)	9/70 (12·9)	1·26 (0·30–4·64)	0·71
Kiwifruit	3/32 (9·4)	4/70 (5·7)	1·71 (0·23–10·74)	0·50
Watermelon	2/32 (6·3)	3/70 (4·3)	1·49 (0·12–13·65)	0·67
Mandarins	2/32 (6·3)	3/70 (4·3)	1·49 (0·12–13·65)	0·67
Grapes	5/32 (15·6)	9/70 (12·9)	1·26 (0·30–4·64)	0·71
Shellfish (raw or cooked)	16/36 (44·4)	29/70 (41·4)	1·13 (0·46–2·75)	0·77
Shellfish (raw)	10/34 (29·4)	9/66 (13·6)	2·64 (0·84–8·32)	0·06
Commercial vegetable salad, raw or cooked	16/36 (44·4)	24/70 (34·3)	1·53 (0·62–3·78)	0·31
Commercial vegetable salad, raw	14/34 (41·2)	20/66 (30·3)	1·61 (0·62–4·20)	0·28
Commercially prepared bakery item	19/35 (54·3)	28/69 (40·6)	1·74 (0·70–4·33)	0·19
Ready-to-eat meat product	27/35 (77·1)	54/71 (76·1)	1·06 (0·37–3·12)	0·90
Non-town supply water	13/37 (35·1)	22/71 (31·0)	1·21 (0·47–3·06)	0·66
Overseas travel during incubation period in case	8/39 (20·5)	8/71 (11·3)	2·03 (0·61–6·76)	0·19

* Denominator excludes participants who answered 'don't know' to question.

2·8 times the average for the same month over the preceding 4 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 fertilize the orchard.

Virological investigation

Clinical specimens

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

Food samples

HAV was detected by reverse transcriptase polymerase chain reaction in 3/6 samples of stored frozen blueberries from the coolstore.

Confirmation of HAV identity

HAV-positive faecal and blueberry specimens were confirmed by DNA hybridization of PCR products. Further confirmation of HAV presence was obtained by DNA sequencing of PCR products from 1/3 HAV-positive blueberry samples and 3/5 HAV-positive faecal specimens. Technical difficulties with HAV sequences derived from faecal specimens limited

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the ability to fully characterize the HAV strains. Where possible, a consensus sequence for each specimen was obtained and compared with HAV sequences deposited in the NCBI Genbank database. More than 92% similarity was observed between 170 bp sequence fragments from the faecal and blueberry specimens, and HAV sequences in the database. However, it was not possible to assign the HAV strains to any of the seven recognized HAV genotypes [9] because our sequences were from the VP3 region rather than the well characterized VP1-2A region.

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 reported to us by these countries as being associated with blueberry consumption. The 6-week interval between the national and international warnings was due to reluctance of exporters to provide information and inadequate records leading to poor product traceability.

Food safety programmes (based on hazard analysis critical control points [HACCP]) covering all berry fruit production and processing are being developed by berry production and marketing organizations. 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 outbreak of hepatitis A associated with the consumption of contaminated blueberries in New Zealand. 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 with hepatitis who attended the orchard. He may (along with other pickers) have been the source of the outbreak, or he may have himself been infected by blueberries which had been contaminated by pickers or by groundwater. If the outbreak was due to contamination by hand it could have been prevented by adequate hand hygiene. 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 [10].

Outbreaks of hepatitis A have been associated with consumption of lettuce [11], frozen strawberries [12, 13] and frozen raspberries [14, 15]. The multistate outbreak associated with strawberries [12] 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 [16].

Despite nation-wide distribution of 14 tonnes of product from the orchard, only 27 cases were identified who reported raw blueberry consumption (Fig. 1). This suggests that contamination was at a low level and/or not uniform. A similar phenomenon has been noted in other outbreaks [12, 13]. 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 [17], 15% of them are unlisted in public directories (Karen Witten, personal communication, Alcohol and Public Health Research Unit). Cases and controls differed in their ethnic distribution.

Recall of dietary history over 4–8 weeks is of questionable accuracy and cases are more likely to remember what they ate than controls (recall bias).

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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 [3] in the berry fruit industry.

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