Participant-collected, Mail-delivered Oral Fluid Specimens Can Replace Traditional Serosurveys

A Demonstration-of-feasibility Survey of Hepatitis A Virus-specific Antibodies in Adults

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ABSTRACT

Background: Although population-based serosurveys offer an optimal measure of cumulative infection rates, they are seldom performed due to high cost and complex logistics. Use of participant self-collected oral fluid as a diagnostic specimen and mail for specimen delivery has the potential of generating reliable, population-representative data at limited cost.

Methods: A survey of oral fluid HAV-specific immunoglobulin G (an indicator of past HAV infection) was undertaken in a provincially representative sample of 20-39 year olds as a pilot study. A provincial administrative database served as the sampling frame. Potential participants were invited by mail to collect oral fluid and complete a questionnaire at home and return both by mail. Additional telephone prompting was directed at slow responders. Oral fluid was tested using a validated ELISA.

Results: From among 2,448 potential participants, contact by mail or telephone was made with 1,009 eligible subjects; 59% (585) participated. Materials withstood mailing and the quality of self-collected specimens was excellent. A positive test result was found in 22.1% overall and in 15.7% of self-reported non-vaccinated subjects. Among Canadian-born, non-vaccinated individuals, the positive test rate increased progressively from 1.2% (95% CI: 0-6.3) in 20-24 year olds to 16.4% (95% CI: 9.5-23.3) in 35-39 year olds. Antibody prevalence was higher among Canadian-born non-immunized 20-29 year olds who reported travel to developing countries (33.3%, 95% CI: 11.6-55.1) than in non-travellers (2.5%, 95% CI: 0.7-6.2).

Conclusions: Mail-based population surveys of infection markers in oral fluid are feasible provided an appropriate sampling frame is used. This survey revealed a high anti-HAV antibody prevalence in young Canadian adults, increasing with age and travel to developing countries.

MeSH terms: Hepatitis A; antibodies, viral; prevalence; surveys; saliva

La traduction du résumé se trouve à la fin de l'article.

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Infectious disease surveillance based on passive reporting of newly diagnosed cases often does not reflect the true rate of infections. Estimating the population burden of non-reportable infections is even more difficult and error-prone. The resulting lack of reliable epidemiological information hampers development of optimal public health strategies. Population-based serosurveys of infectious disease markers offer accurate estimates of the cumulative infection risk but they are costly and difficult to organize. When attempted, serosurveys are often of limited scale and aimed at populations of convenience. Epidemiological investigations using non-invasive, participant-collected specimens that are mailed to the laboratory have the potential to provide accurate population-based data with less cost and effort than a traditional serosurvey, making large-scale investigations feasible.^{1,2} To the authors' knowledge, such studies have never been attempted in North America.

In British Columbia, a discrepancy between hepatitis A incidence rates³ based on reported cases and available anti-HAV prevalence data⁴ (authors' unpublished data) called for a population-based, province-wide investigation of past infection rates. Armed with a suitably accurate assay,⁵ we undertook a pilot oral fluid/mail-based provincial survey of anti-HAV among young adults.

METHODS

Population and sample size

Our sampling frame was the Client Registry of the British Columbia Ministry of Health Services, an administrative database that includes contact information on almost all provincial permanent residents. Registry custodians performed random sampling in two age strata (20-29 and 30-39 years) and supplied contact information on 2,488 potential study participants. A sample size of 200 individuals per age stratum was estimated to provide acceptable accuracy, with the 95% confidence interval less than $\pm 10\%$ at an anti-HAV prevalence of 25%.

Survey mechanics

All potential participants were mailed a package containing information about the study in English, including a consent/refusal form, one-page questionnaire and tools required for collecting and

returning oral fluid to the laboratory. Procedures were initially evaluated with a test panel of 25 adults who received the prototype invitation package. Their feedback was used to refine the materials. Consent forms, questionnaires and specimen containers were linked by pre-applied bar-coded labels. Questionnaires and specimen containers bore no personal identifiers. A tracking program was used to monitor each potential participant to the point of specimen receipt or abandonment of attempts to contact. Two weeks after the mail-out, non-responders were to be contacted by telephone to encourage participation and clarify their intentions. At least three separate attempts at different times of day were made to establish telephone contact with non-responders.

Oral fluid collection

Oral fluid was collected using the OmnisAL (Saliva Diagnostic Systems, Medford, NY, USA) device consisting of a fluid-absorbing pad and handle with saturation colour indicator and transportation tube. The pad was held under the tongue until saturated with mouth fluid, then transferred to a transportation tube containing preserving buffer. This preserves non-refrigerated specimens for several days without compromising suitability for antibody analysis.^{6,7} Upon arrival at the laboratory, specimens were recovered using standard serum filters, aliquoted and kept at -70°C until assayed.

Demographic data collection

Participants were asked to complete a one-page questionnaire and return it along with the oral fluid sample. This instrument was validated previously⁸⁻¹⁰ and collected the following information: birth date and gender; country of birth and, if relevant, age of immigration to Canada; the first three digits of their home postal code as a community-level geolocator; any personal or family history of hepatitis A infection, personal history of hepatitis A immunization; and past extensive travel to developing countries. We did not pose questions regarding racial group, injection drug use, sexual practices or involvement in the sex trade.

Laboratory methods

Specimens were screened for total immunoglobulin G (IgG) using a capture

TABLE I

Selected Demographic Characteristics of Anti-HAV IgG-positive and -negative Participants

	Total	Number of Participants		95% CI
		Anti-HAV Positive	% Positive	
All Participants	585	129	22.1	18.7-25.4
Male	224	45	20.1	14.8-25.3
Female	361	84	23.3	18.9-27.6
20-29 years old	266	54	20.3	15.5-25.1
30-39 years old	319	75	23.5	18.9-28.2
Hepatitis A-vaccinated after 1994	69	48	69.6	58.7-80.4
Hepatitis A non-vaccinated	516	81	15.7	12.6-18.8
Foreign-born	107	45	42.1	32.7-51.4
Canadian-born	409	36	8.8	6.1-11.6
20-29 years old	182	10	5.5	2.2-8.8
30-39 years old	227	26	11.5	7.3-15.6
Travellers	49	10	20.4	9.1-31.7
Non-travellers	358	26	7.3	4.6-10.0

quantitative ELISA that is calibrated against Human Immunoglobulin Reference Standard. Samples adequate by volume (>500µL) and total IgG content (>0.1 mg/L) were tested for HAV-specific IgG using an oral fluid ELISA previously shown to be 99% sensitive and specific in differentiating between immune and susceptible individuals when compared to conventional serum-based testing.⁵

Statistical analysis

Demographic data and test results were entered into Microsoft Access using dual data entry and pre-programmed consistency checks to minimize transcription errors.

Categorical data were compared using Chi square and Fisher's exact tests, and 95% confidence intervals were calculated using standard methods.

Ethical approval

The study protocol was approved by the Clinical Research Ethics Board of the University of British Columbia and by the Children's and Women's Health Centre of B.C. Research Review Committee.

RESULTS

Enrollment outcome

We were able to contact 1,177 of 2,488 (47.3%) potential study participants. Reasons for non-contact included incorrect contact information (76%) or inability to make contact by telephone after several attempts (24%). Among contacted prospects, 169 were not eligible for study participation. Among the remaining 1,009 contacted and eligible subjects, 215 (21.3%) refused to participate. The refusal

rate was similar for both age strata (19.6%-22.9%). Ultimately, 585 subjects (58.0% of contactable eligible persons) returned an adequate sample (as below) and completed questionnaire, 182 individuals (18.0%) who indicated intent to submit a sample did not do so, and 27 (2.7%) returned an inadequate sample or incomplete questionnaire. Of the 612 specimens received by the laboratory, only 140 (22.9%) were submitted without telephone prompting. Selected demographic characteristics of the 585 compliant study participants are shown in Table I.

Quality of submitted specimens

Among 612 submitted specimens, 34 (5.6%) were not suitable for testing (27 transportation tubes leaked, 1 tube was cracked, 4 were missing the collecting pad and 2 had an implausibly large volume). These 34 subjects were asked to provide another specimen but only 12 complied, so ultimately 96.4% of submitted specimens were acceptable. All 590 specimens contained a sufficient amount of total IgG.

Prevalence of HAV specific antibody

A positive test was less common among Canadian-born compared to foreign-born individuals (see Table I). Among non-vaccinated Canadian-born subjects, antibody was more prevalent among individuals in their thirties than in their twenties. As illustrated in Figure 1, past HAV infection was rare in subjects 20-24 years old but more common in those aged 35 to 39. Among non-vaccinated, Canadian-born participants, past HAV infection was more common among those who reported prior extensive travel to developing countries

Figure 1. Anti-HAV IgG among Canadian-born, non-vaccinated individuals according to age.

than among those who did not ($p=0.002$). Anti-HAV was more common in travellers aged 20 to 29 (6/18, 33.3%, 95% CI: 11.6-55.1) than among non-travellers of this age (4/163, 2.5%, 95% CI: 0.7-6.2). However, among participants aged 30 to 39, the rate of past infection was similar among travellers and non-travellers (12.9%, 95% CI: 3.6-29.8 versus 11.3%, 95% CI: 6.8-15.7).

DISCUSSION

This study demonstrates that a large-scale, population-based epidemiological survey using oral fluid as the diagnostic medium and regular mail to deliver survey tools to participants and specimens to the laboratory is feasible in North America provided that a sampling frame with correct contact information for sampled individuals is used. This approach is relatively inexpensive: this survey was successfully completed within a CAD \$100,000 operating budget. Although 59% of eligible, contacted subjects participated in the survey, the overall recruiting success was low (25.2%) mainly because contact information in the administrative database that we used as a sampling frame was incorrect nearly half the time. An accurate alternative sampling frame would improve the results and

reduce effort and cost. Oral fluid collection kits survived postal handling well – only 1 transportation tube cracked out of over 600 returned devices. More than 96% of specimens were adequate for testing.

The main requirement for oral fluid/mail-based epidemiological investigations is availability of sensitive immunoassays. Concentrations of antigen-specific IgG in oral fluid are much lower than in serum,^{11,12} so assays developed for serum are unsuitable for oral fluid which requires specially designed assays or recalibration (if possible) of serum assays. The range of suitably sensitive and specific tests for oral fluid analysis is currently limited, but reports have included assays for hepatitis A, B, and C, HIV, measles, rubella and mumps.^{13,14}

To increase the co-operation rate of potential study participants, we employed a number of techniques known to improve responses to questionnaire/mail-based surveys such as use of university letterhead on communications, promoting participation through descriptive messages in provincial and local media (tv, radio, press), offering lottery-based small prizes to participants, and offering to confidentially communicate individual test results to interested participants. The last incentive was thought to play a substantial role as almost

all participants requested their test result. Despite these incentives, fewer than 25% of those who provided a sample did so within two weeks of being approached and without telephone prompting. Although telephone contact was essential to encourage participation in this survey, almost one fifth of those who expressed willingness to participate during the telephone conversation never provided a specimen.

A limitation of our data is that only about one quarter of the originally selected sample participated in the study. Factors that limited participation could have created bias. Most non-participants had incorrect addresses, precluding contact. Perhaps young people who relocate are at higher risk but there are no reports suggesting this, with the exception of travellers to hepatitis A-endemic areas. The next largest group of non-participants could not be reached by telephone. Use of caller identification may have been a factor in non-response. Perhaps people who are difficult to reach by telephone are at greater or lower hepatitis risk, but again there is no evidence available to support such a hypothesis. Some biases might have been introduced by omitting smaller groups without a fixed address and telephone service, such as street youth and the homeless. Those who refused to participate or did not provide a specimen for testing are perhaps at higher risk. As our study materials were only in English, it is likely that language barriers prevented some immigrants from participating. However, the proportion of immigrants among survey participants was similar to the proportion of immigrants among British Columbians of this age. We do not believe that any of these processes of sample narrowing had a major effect on the validity of results.

The overall prevalence rate (22.1%) of anti-HAV observed in this study was similar to the rate observed among BC women 15-44 years old (23.5%)⁴ and among 20-39 year old international travellers attending a local pre-travel clinic (16.2%) (authors' unpublished data). Nearly 12% of young adults reported previous vaccination for hepatitis A, even though distribution of vaccine has been limited to privately purchased vaccine through travel clinics, and a public health strategy that targets specified high-risk groups. Anti-HAV was detected in 69.6% of vaccinees, who have

lower serum antibody concentrations than follow natural infection. The relatively high prevalence of anti-HAV among non-vaccinated individuals resulted mainly from individuals born outside Canada, as the rate in Canadian-born subjects was only 8.8%. The greater prevalence of anti-HAV in Canadian-born individuals in their late thirties than in their early twenties is consistent with our earlier reports^{8,10} that hepatitis A is relatively uncommon among children and suggests that young adults are at substantial risk. Our data also support earlier reports^{10,15} that foreign travel is an important risk factor for Canadians. While the number of anti-HAV-positive individuals and the number reporting travel to the developing world for more than 2 weeks at a time were relatively few, it is worth noting that 60% of Canadian-born, non-vaccinated individuals with anti-HAV reported such travel. The reasons why a similar association was not present in the older cohort are unclear, but might simply reflect a shift in popularity of certain travel destinations or activities posing a higher risk of hepatitis A infection among young people over time. Exotic travel destinations such as India, Nepal and South America may be more commonly visited by young adults (and usually on a limited budget) today than a decade or two earlier.

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RÉSUMÉ

Contexte : Les enquêtes sérologiques représentatives sont le meilleur moyen de mesurer les taux d'infection cumulatifs, mais elles sont rarement exécutées en raison de leur coût élevé et de leur complexité logistique. L'utilisation d'échantillons de liquide oral autoprélevés et envoyés par la poste pour évaluation diagnostique pourrait peut-être produire des données fiables et représentatives à moindre coût.

Méthode : Nous avons enquêté sur l'immunoglobuline G salivaire propre au VHA (un indicateur d'infection à VHA antérieure) auprès d'un échantillon représentatif provincial de sujets de 20 à 39 ans dans le cadre d'une étude pilote. Une base de données administratives provinciales nous a servi de base de sondage. Les participants éventuels ont été invités par la poste à prélever de la salive, à remplir un questionnaire à domicile et à retourner l'échantillon et le questionnaire par la poste. Les retardataires ont été relancés par téléphone. Les échantillons de liquide oral ont été testés par dosage immunoenzymatique validé (technique ELISA).

Résultats : Sur les 2 448 participants possibles, 1 009 sujets admissibles ont été contactés par la poste ou par téléphone, et 59 % (585) ont participé à l'étude. Le matériel utilisé a résisté à l'envoi postal, et les échantillons autoprélevés étaient d'excellente qualité. Nous avons obtenu des tests positifs pour 22,1 % de tous les répondants et pour 15,7 % des sujets s'étant déclarés non vaccinés. Parmi les personnes non vaccinées nées au Canada, le taux de tests positifs augmentait progressivement avec l'âge, soit de 1,2 % (IC de 95 % = 0-6,3) chez les 20 à 24 ans à 16,4 % (IC de 95 % = 9,5-23,3) chez les 35 à 39 ans. La prévalence d'anticorps était plus élevée chez les personnes de 20 à 29 ans non vaccinées et nées au Canada disant avoir voyagé dans des pays en développement (33,3 %, IC de 95 % = 11,6-55,1) que chez les non-voyageurs (2,5 %, IC de 95 % = 0,7-6,2).

Conclusions : Les enquêtes représentatives effectuées par la poste sont faisables pour déceler les infections dans des échantillons de liquide oral, à condition d'utiliser une base de sondage appropriée. Notre enquête a mis au jour une prévalence élevée d'anticorps anti-VHA chez les jeunes adultes canadiens, prévalence qui s'accroît avec l'âge et les voyages dans des pays en développement.