Integrating laboratory and epidemiological techniques for population-based surveillance of HIV strains and drug resistance in Canada

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HIV is among the most genetically variable of human pathogens. Two major factors contribute to this genetic diversity: the error-prone activity of reverse transcriptase, which is estimated to introduce an average of one error/genome/replication cycle (1), and recombination, which occurs at a rate of about 2%/kilobase/replication cycle (2).

With the advent of international collaborations using powerful new tools that allow for the analyses of nucleotide sequence information, it became apparent that the initial classification of HIV into HIV-1 and HIV-2 based on geographic distribution was inadequate. We now recognize that HIV-1 can be divided into three major phylogenetic groups: 'M' (major), 'O' (outlier) and more recently, 'N' (new). The vast majority of isolates cluster in the M group. Based on sequencing the envelope gene, *env*, 10 phylogenetic subtypes (A to J) have been identified within this group, with subtypes A to E (also referred to as the circulating recombinant A/E) being the most common (3). The general pattern of subtype distribution by geographic location is shown in Table 1.

The second major group of HIV-1, group O, is found mainly in Cameroon and Gabon, and differs from the M group by as many as 50% of residues (4). The N group of HIV-1 was isolated in Cameroon, with genetic characteristics of both the simian immunodeficiency virus and HIV-1 (M group) (5).

Although there has been no systematic surveillance for genetic diversity of HIV strains in Canada, existing studies on high risk populations suggest that HIV-1 subtype B is the most common subtype found in this country. Bernier et al (6) have conducted analyses on HIV-1 sequence diversity among 17 infected injection drug users (IDUs) and among five men who have sex with men (MSM) residing in Montreal, and all sequences were of HIV-1 subtype B. As a part of an outbreak study in Newfoundland, Montpetit et al (7) analyzed serological samples from 31 HIV-positive persons of both sexes, comprising approximately 25% of known HIV-positive persons in the province, to determine the extent of HIV-1 subtype variation (7). All samples tested were of HIV-1 subtype B. Strain analysis has been carried out on samples from 13 MSM, two IDUs and two heterosexuals, recruited through POLARIS in Ontario (8). All have been found to be subtype B. The British Columbia Centre for Excellence in HIV/AIDS in Vancouver, British Columbia, has conducted genetic analysis of HIV linked to VIDUS (9). All 64 IDUs tested were infected with subtype B.

Despite the predominance of HIV-1 subtype B, non-B subtypes have also been reported in Canada. As early as 1995, HIV-1 subtype A was identified in an African-born male, who moved to Canada in 1983 (10). Studies by the British Columbia Centre for Excellence in HIV/AIDS suggest that non-B subtypes represent at least 4% of HIV infections among

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individuals starting therapy (11). HIV-1 subtypes A, C, and D have been identified. Non-B subtypes were associated with poor outcomes upon initiation of treatment (12).

THE CANADIAN HIV STRAIN AND DRUG RESISTANCE SURVEILLANCE PROGRAM

The Canadian HIV Strain and Drug Resistance Surveillance Program (CHSDRSP), based in the Bureau of HIV/AIDS, STD and TB, Laboratory Centre for Disease Control, Health Canada, Ottawa, Ontario, was initiated to characterize and monitor the genetic diversity of the HIV epidemic in Canada. CHSDRSP consists of two principle components: laboratory-based genetic information and epidemiological information. It is designed to serve as an integrated mechanism for the analysis of HIV genetic characteristics as they relate to the epidemiology of HIV, addressing the concerns of public health authorities, primary care physicians and researchers. The four goals of CHSDRSP are as follows.

To assess circulating strains

Strain variation in Canada and around the world needs to be identified and well understood to develop effective vaccines, to understand the transmissibility of strains and to understand the pathogenicity of the strains. Because vaccines may be effective against certain subtypes only, it is important to know the distribution of the viral subtypes in Canada to determine the effectiveness of proposed vaccines and to target future vaccine development and testing.

While there is no clear evidence of a correlation between HIV-1 subtype and the biological behaviour of the virus, some studies suggest the different subtypes of HIV may have different transmission patterns. For example, in Thailand, although two different HIV-1 subtypes, E and B, were introduced at approximately the same time, the proportion of subtype E has increased in most population groups (13). This and other observations showing changes in the proportion and distribution of subtypes over time suggest that certain non-B subtypes, including subtype E, may be transmitted more effectively during heterosexual intercourse (14). If confirmed, this would be cause for concern because the introduction of subtype E to Canada could significantly change the nature of the HIV epidemic in this country.

Preliminary work by Alexander et al (15) indicates that B and non-B HIV subtypes respond similarly to antiretroviral treatment. The effects of viral subtype on response to therapy and the public health implications are also of special interest.

To assess genetic markers for drug resistance

Monitoring and assessing the patterns of primary drug resistance, such as is done for resistant forms of other sexually transmitted diseases including gonorrhea, can enhance the utility of CHSDRSP. Determining the extent of resistant viral genotypes among those recently diagnosed with HIV infection would be useful in developing treatment strategies and new interventions to prevent the spread of HIV-1 among the Canadian population.

TABLE 1
Geographic distribution of group 'M' subtypes of HIV-1

HIV-1 (M) subtype	Geographic area
A, D	Central and East Africa
B	Americas, Europe, India, Thailand
C	India, South and Central Africa
E	Central African Republic, India, Thailand
F	Brazil, Congo, Eastern Europe
G	Gabon, Taiwan, Congo
H	Congo, Gabon
I	Cyprus
J	Congo

To assess patterns of transmission

Given the impact of international travel and migration on the spread of HIV, it is likely that non-B subtypes will continue to be introduced into Canada. If unusual strains are detected, this could contribute to the knowledge of infections acquired outside of the country. Genetic linkages could be done in a community or remote area to determine what proportion of HIV is brought in from outside the area. Combining strain surveillance data with enhanced information gathering from individuals could also be very useful in investigating transmission patterns. Additionally, for special studies outside the strict parameters of surveillance, genetic sequencing could establish the probability that two or more individuals share a common virus.

To enhance the safety of the blood supply

To enhance the safety of the blood supply, HIV screening tests need to detect reliably the circulating strains in this country. The precedent for this goal was the discovery of HIV-2 and highly divergent group O strains of HIV-1 which required modification of some serological screening tests by adding these new antigens to ensure detection. CHSDRSP will monitor the circulating strains of HIV-1 to facilitate the discovery of new HIV variants that might not be detected by current HIV screening tests. Based on the knowledge of circulating HIV types, and vigorous laboratory testing of individuals with unusual clinical presentations and of samples showing unusual serological, polymerase chain reaction (PCR) or viral culture results, modifications can be made in current tests to ensure that all HIV-1 positive persons in Canada are detected upon testing. There are many obvious implications of reliably screening and detecting new HIV strains, including protecting the blood supply, ensuring accurate diagnosis and understanding HIV transmission patterns.

CHSDRSP INITIATIVES

Laboratory References Services, Bureau of HIV/AIDS, STD and TB, LCDC

Provincial health laboratories (PHLs) and Canadian Blood Service are involved in HIV testing of individuals, and as such are key partners to CHSDRSP. A number of factors that result in unusual serological responses are relatively well established (seroconversion and cross-reactivity to HIV-2) and are

TABLE 2
HIV-1 subtype distribution of nine "difficult to diagnose" samples submitted to the National Laboratory for HIV Genetics for viral subtyping

Province	Subtype	Year sample submitted to NLHG
Alberta	B	1998
Manitoba	C	1998
	C	1999
	B	1999
Ontario	A	1998
	B	1999
	A	1999
Nova Scotia	C	1998
Newfoundland	A	1999

NLHG National Laboratory for HIV Genetics

recognized by the testing laboratories. Other conditions that are less well understood can also cause unusual serological test results. These samples may show cross-reactivity or represent divergent strains, as seen in 1993 with the failure of some diagnostic kits in France to detect HIV-1 subtype O (16).

The National Laboratory for HIV Genetics (NLHG) and National Laboratory for HIV References Services (NLHRS) serve PHLs by testing and identifying samples showing unusual serological, PCR or viral culture testing results. This ongoing partnership serves as an additional surveillance arm of CHSDRSP and is crucial for monitoring divergent HIV strains in Canada.

In the past two years, five provinces have submitted serological samples to the NLHG from individuals that were difficult to diagnose. These samples were analyzed using the techniques mentioned below, and the subtypes were determined. The results of these analyses are shown in Table 2. The majority of the samples submitted were of subtypes A and C. Of note, two subtype A samples and one subtype C sample were obtained from individuals born or infected in Africa.

CHSDRSP Pilot Study

At a national consensus meeting in January 1998 to discuss the feasibility of the goals of CHSDRSP, working groups including federal, provincial and territorial stakeholders recommended piloting the surveillance methodologies at one or more sites before implementing CHSDRSP nationwide. The five sites included in this pilot phase were British Columbia, Alberta, Saskatchewan, Manitoba and Newfoundland. This section describes interim results from subtype analysis of archived samples taken for HIV diagnosis or viral load testing in the CHSDRSP pilot sites.

Methodology – ***Laboratory:*** Archived sera collected from newly diagnosed HIV infected persons or plasma collected from persons coming for viral load testing were sent by the PHLs at the pilot sites to the NLHG at LCDC for subtype analysis.

The protocol for HIV-1 subtyping was as follows. Viral particles in 200 μL of serum were pelleted at 21,000 × *g* for 1 h at 4°C, the supernatant aspirated and the RNA from the viral pellet isolated using the NucliSens silica extraction system (Organon Teknika, Toronto, Ontario) derived from Boom et al (17). Single tube reverse transcription PCR (RT-PCR) were performed using *env* specific primers ED3 (HXB2 position 5956-5985) and ED14 (HXB2 position 7960-7931) with the Calypso RT-PCR kit (Bio/Can, Mississauga, Ontario). DNA from cells and dried blood spots were amplified by PCR also using these *env* specific primers. Secondary, nested PCR reactions, using 5 μL of primary PCR product, were performed with primers ED5 (HXB2 position 6556-6581) and ED12 (HXB2 position 7822-7792) in QIAgen Master Mix (Mississauga, Ontario). Simultaneous bidirectional sequencing reactions were prepared using the ThermoSequenase kit (Amersham, Montreal, Quebec) and IRD-labelled primers ES7 (HXB2 position 7001-7020), and ES8 (HXB2 position 7667-7647) yielding a complete double stranded sequence for 650 bases of the envelope gene (V2-V5 region). The reactions were run on a LI-COR 4200L, and the results were assembled and analyzed using AlignIR (Li-Cor, Lincoln, Nebraska), Sequencher (Gene Codes, Ann Arbor, Michigan) and software developed by NLHG.

Epidemiology: In addition to the laboratory specimen, corresponding epidemiological information was also collected and sent to the Division of HIV Epidemiology, Bureau of HIV/AIDS, STD & TB. The data included information on age, sex, ethnicity, year of positive test, city where test was conducted, type of serological specimen collected and risk factors associated with HIV infection. However, because the majority of data was extracted retrospectively from medical records, not all the information mentioned above was available for each sample. Subtype information for each sample from the NLHG was linked to the corresponding epidemiological information using the unique specimen identifiers, and further analysis was con-

TABLE 3
Distribution of HIV-1 subtypes from the Canadian HIV Strain and Drug Resistance Surveillance Program pilot study by province

Province	Subtype A n (%)	B n (%)	C n (%)	D n (%)	A/B n (%)	A/E n (%)	Total (%)
British Columbia	5 (4.59)	92 (84.4)	9 (8.26)	1 (0.92)	1 (0.92)	1 (0.92)	109 (100)
Alberta	–	10 (90.9)	1 (9.1)	–	–	–	11 (100)
Saskatchewan	4 (3.88)	95 (92.2)	4 (3.88)	–	–	–	103 (100)
Manitoba	–	25 (96.2)	1 (3.85)	–	–	–	26 (100)
Newfoundland	–	20 (100)	–	–	–	–	20 (100)
Total	9 (3.35)	242 (90)	15 (5.58)	1 (0.37)	1 (0.37)	1 (0.37)	269 (100)

TABLE 4
Epidemiological information for non-B subtypes from British Columbia (Canadian HIV Strain and Drug Resistance Surveillance Program pilot site)

Subtype	Sex	Risk factor	Year of first positive HIV test
A	Male	MSM/IDU	1996
	Male	IDU	1996
	Male	MSM	1997
	Female	WSW	1997
	Male	Heterosexual sex	1998
C	Male	MSM/IDU	1996
	Female	Heterosexual sex	1996
	Male	Heterosexual sex	1996
	Male	IDU	1996
	Male	Heterosexual sex	1996
	Male	IDU	1996
	Male	MSM	1996
	Male	MSM	1996
	Male	Heterosexual sex	1998
D	Female	Heterosexual sex	1998
A/B	Male	MSM	1996
A/E	Male	IDU	1997

IDU Injection drug user; MSM Men who have sex with men; WSW Women who have sex with women

TABLE 5
Epidemiological data of HIV-1 B versus non-B subtype for British Columbia (Canadian HIV Strain and Drug Resistance Surveillance Program pilot site)

	Subtype B n=92, n (%)	Non-B subtype n=17, n (%)	P
Sex			
Female	21 (22.8)	3 (17.6)	0.45
Male	70 (76.1)	14 (82.4)	
Unknown	1 (1.09)		
Risk factor			
MSM[†]	23 (25)	2 (11.8)	0.27
MSM/IDU	2 (2.17)	2 (11.8)	
Heterosexual sex	26 (28.3)	6 (35.3)	
IDU	38 (41.3)	6 (35.3)	
Other[‡]	3 (3.26)	1 (5.88)	
Mean age (years ± SD)	40.7±9.9	35.4±8.1	0.04*
Year of first positive HIV test			
1996	21 (22.8)	11 (64.7)	0.003*
1997	22 (23.9)	3 (17.6)	
1998	25 (27.2)	3 (17.6)	
1999	24 (26.1)	0 (0)	
Geographic area of residence			
Vancouver city	71 (78.9)	15 (88.2)	0.79
Outside metropolitian Vancouver	19 (21.1)	2 (11.8)	
Ethnicity			
White	58 (63)	8 (47.1)	0.32
Aboriginal	20 (21.7)	4 (23.5)	
Other[§]	14 (15.2)	5 (29.4)	

**P indicates significant difference between HIV-1 B and non-B subtypes using univariate analysis. [†]MSM includes men who have sex with men (MSM)/intravenous drug users (IDUs). Two MSM/IDUs each were infected with HIV-1 subtype B and non-B, respectively. [‡]'Other' risk categories include women who have sex with women (WSW), occupational exposure, male prostitute and infection with blood or blood products. [§]'Other' ethnicities include Asian (n=11), Black (n=2) and Latin American (n=3)*

ducted using the statistical packages SPSS (SPSS Inc, Chicago, Illinois) and STATA (STATA Corporation, College Station, Texas).

Results: Of the 269 samples for which subtype analysis has been completed, 109 (40.5%) were from British Columbia, 11 (4.1%) were from Alberta, 103 (38.3%) were from Saskatchewan, 26 (9.7%) were from Manitoba and 20 (7.4%) were from Newfoundland.

Two hundred and forty-two or 90% of the samples belonged to HIV-1 subtype B. Of the remaining 27 samples, nine (3.3%) were of subtype A, 15 (5.6%) were of subtype C, one (0.4%) was of subtype D and one each (0.8%) were of recombinant subtypes A/E and A/B, respectively (Table 3).

The preliminary results reported above indicate geographic variation in the distribution of HIV-1 non-B subtypes. Whereas all 20 samples from Newfoundland were identified as subtype B, 15.6% of samples from British Columbia belonged to non-B subtypes. British Columbia also had the greatest genetic variation among the non-B HIV-1 subtypes (Table 3). Among the other pilot sites, the proportion of non-B subtypes was approximately 9.1%, 7.8%, and 3.9%, for Alberta, Saskatchewan and Manitoba, respectively. A more in-depth analysis of strains for each province is presented below. It should be noted that sample sizes in this interim analysis are small for some provinces and are not representative of Canada as a whole. It should also be noted that this report provides information only for those who were tested and diagnosed with HIV-1, and does not represent the total number of persons infected with HIV-1 in Canada.

HIV-1 subtypes for CHSDRSP samples from British Columbia: Stored serum specimen from individuals newly diagnosed with HIV in 1996, 1997, 1998, and up to March 15, 1999 were included in this pilot study. The samples represent between 4% to 6% of HIV-positive tests reported by British Columbia to the Division of HIV Surveillance, Bureau of HIV, STD and TB for each year (18), and were representative of exposure categories of those who were diagnosed with HIV for each year.

Ninety-two or 84.4% of the 109 samples from British Columbia that have been subtyped belonged to HIV-1 subtype B (Table 3). Of the 17 non-B subtypes, five (4.6%) belonged to subtype A, nine (8.3%) were subtype C, one (0.9%) was subtype D, and one each were of recombinant subtypes A/B and A/E. Additional epidemiological information from individuals infected with a non-B subtypes is shown in Table 4.

A comparison of epidemiological information of the HIV-1 subtype B and the non-B samples is shown in Table 5. Univariate analyses indicated no significant difference in sex or risk category between B and the non-B subtypes. Among the sample population, those infected with a non-B subtype tended to be significantly younger (mean age 35.4 years) than those infected with the HIV-1 subtype B (mean age 40.7, P=0.04).

Univariate analysis also indicated a significant difference

TABLE 6
Epidemiological profiles of subtype B samples from Alberta, Manitoba and Newfoundland (Canadian HIV Strain and Drug Resistance Surveillance Program pilot sites)

	Alberta n=10, n (%)	Manitoba n=25, n (%)	Newfoundland n=20, n (%)
Sex			
Female	3 (30%)	5 (20%)	8 (40%)
Male	7 (70%)	20 (80%)	12 (60%)
Risk factor			
MSM	3 (30%)	6 (24%)	4 (20%)
IDU	4 (40%)	9 (36%)	1 (5%)
MSM/IDU	–	1 (4%)	–
Heterosexual sex	3 (30%)	9 (36%)	11 (55%)
Unknown	–	–	4 (20%)
Mean age (years ± SD)	34.5±12.9	34.16±7.9	32.7±6.07
Ethnicity, n (%)			
White	4 (40%)	Not available	20 (100%)
Aboriginal	5 (50%)		
Latin American	1 (10%)		
Year of first positive HIV test			
1985-1989	–	1 (4%)	3 (15%)
1990-1994	–	3 (12%)	12 (60%)
1995	–	2 (8%)	1 (5%)
1996	–	2 (8%)	–
1997	–	–	1 (5%)
1998	9 (90%)	10 (40%)	1 (5%)
Unknown	1 (10%)	7 (28%)	2 (10%)

IDU Injection drug user; MSM Men who have sex with men

between B and non-B subtypes with respect to year of first testing positive for HIV (P=0.003), with the majority of non-B samples (11 of 17) in this interim analysis being diagnosed in 1996. There was no significant difference in subtype outcome among those diagnosed with HIV with respect to geographic residence or ethnicity. There were relatively more non-B subtypes found within metropolitan Vancouver and among ethnic groups other than White or Aboriginal persons, but neither association was statistically significant. Because of the low sample size in this interim analysis and the associated low statistical power, the results do not clearly differentiate between no true association and an association that simply was not detected by this analysis. It should be noted that two individuals in this group of samples identified themselves as Black, and both were infected with non-B subtypes, one with subtype A and the other with subtype D. Three of the 11 individuals who identified themselves as Asian were infected with non-B subtypes, two with subtype A and one with subtype C.

Logistic regression to determine the simultaneous effects among variables indicated that age and year of first testing positive for HIV were significantly associated with a non-B outcome (P=0.009 and P=0.0003, respectively) in the sampled population. Compared with the B-subtype, the likelihood of a non-B subtype among those diagnosed with HIV was 1.11 times greater for every year decrease in age. The likelihood of infection with a non-B subtype in the sample population was 12.9 times higher among those diagnosed in 1996 compared with the three subsequent years combined. Ethnicity was of borderline significance in logistic regression; White and Aboriginal groups together were more likely to be diagnosed with B-subtype compared with other ethnic groups, but this association was not statistically significant at the P=0.05 level (P=0.071). Further analysis using a larger sample size is necessary to help elucidate this association.

HIV-1 subtypes for CHSDRSP samples from Alberta: Stored plasma specimens collected from individuals coming for viral load testing were included in this pilot study. The samples represent 9.3% of individuals reported as testing positive for HIV in Alberta during 1998 (18). Ten of the 11 samples (90.9%) for which subtype analysis has been completed belonged to HIV-1 subtype B (Table 3). The epidemiological profile of these individuals is shown in Table 6. The remaining sample was identified as HIV-1 subtype C. This sample was collected from a 30- to 35-year-old female, infected through heterosexual transmission, who had moved to Canada from Africa and who first tested positive for HIV in 1998.

HIV-1 subtypes for CHSDRSP samples from Saskatchewan: Stored samples collected from individuals coming for viral load testing were included in this pilot study. Ninety-five of the 103 samples (92.2%) from Saskatchewan were of HIV-1 subtype B (Table 3). Of the remaining eight samples, four belonged to subtype A (three women and one man) and four to subtype C (three men, one woman). Note that these 103 samples represent 24.8% of HIV diagnoses in Saskatchewan during 1985 to June 30, 1999 (18).

HIV-1 subtypes for CHSDRSP samples from Manitoba: Plasma samples collected from individuals coming for viral load testing, diagnosed with HIV-1 infection from a series of years before 1999, were selected for inclusion in this pilot study. These samples represent 0.85%, 7.1%, 8.3% and 38% of individuals reported as testing positive for HIV in 1985 to 1994, 1995, 1996 and 1998, respectively (18). Of the 26 samples on which subtype analysis was completed, 25 (96.2%) belonged to HIV-1 subtype B (Table 3). The epidemiological profile of these individuals is shown in Table 6. The remaining sample belonged to subtype C. It was collected from a male who was age 25 to 29 years, infected through heterosexual transmission and first tested positive in 1998.

HIV-1 subtypes for CHSDRSP samples from Newfoundland: Specimens collected from individuals coming for viral load testing with a wide variation in date of HIV diagnosis were included in this pilot study. These samples represent 9.6%, 14.3%, 14.3% and 7.1% of individuals testing positive for HIV between 1985 and 1994, 1995, 1997 and 1998, respectively (18). All 20 individuals in the sample population from Newfoundland were infected with HIV-1 subtype B (Table 3). The epidemiological profile of these individuals is shown in Table 6.

DISCUSSION

At the end of 1996, there were approximately 40,000 Canadians living with HIV infection (19). The number of incident HIV infections in Canada for 1996 was approximately 4200,

higher than the average of 2500 to 3000/year from 1989 to 1994 (19). An analysis of the distribution of incident infections by exposure category illustrates a changing face of the HIV epidemic in Canada, where now nearly one-half of all new infections can be attributed to injection drug use (19). Women are increasingly affected as are minority populations.

In addition to the aforementioned changes in the populations infected with HIV, the present results confirm that a significant proportion of non-B HIV-1 subtypes has been introduced into Canada and suggest that there is likely geographic variation in the prevalence of these subtypes. From the sample population analyzed for this interim report, 10% were infected with a non-B subtype, of which 3.3% were of subtype A, 5.6% were of subtype C and 0.4% were of subtype D.

It should be emphasized that the sample population represents those diagnosed with HIV from a variety of years and is not reflective of the total population infected with HIV. Furthermore, these results may not be representative of the respective provinces, with respect to HIV strains that are currently circulating. The percentage of HIV test positive reports represented in the sample population varies between the respective provinces. For example, up to 38% of all positive HIV tests reported for 1998 from Manitoba are represented in this report, whereas only 9.3%, 7.1%, and 5.8% of positive HIV tests reported for 1998 are represented from Alberta, Newfoundland and British Columbia, respectively.

Despite these limitations, our findings are similar to those documented for other countries where subtype B used to predominate. In Sweden, until 1984, the HIV epidemic was almost entirely caused by subtype B; however, by 1993, non-B subtypes A and C, accounted for 30% of all new infections (20). In a pilot study conducted in Germany, eight of 24 recent HIV-1 infections among German nationals (33%) were because of non-B subtypes including subtypes A, C, and E (21). In England, subtypes A, C, D, and A/E have been identified (22), and in Belgium, subtypes A, C, D, F, G, and H were reported in 1996 (23). In the United States, subtype D was identified as early as 1993 (24). Sentinel site surveillance of HIV in the United States has also indicated the introduction of other non-B subtypes into the country. A non-blinded study in 1992 to 1994 at the Bronx-Lebanon Hospital Center (Bronx, New York) found HIV-1 subtype A in two of 43 (5%) patients (25). A follow-up study found that among 91 HIV-1 infected patients, one (1.1%) was infected subtype A and two (2.2%) with subtype F (26). In 1998, the Centers for Disease Control and Prevention, Atlanta, Georgia (CDC) initiated a program for the sentinel surveillance of strain and drug resistance nationwide. Results from this study suggest that 1.6% of newly diagnosed (within the past year) HIV-infected persons, who are at least 18 years of age and have no history of AIDS-defining illnesses according to the 1993 CDC AIDS case definition, are infected with a non-B subtype. Specifically, subtype A has been identified in five persons in a sample population of 321 (27).

While the CHSDRSP reference services and the pilot study have identified HIV-1 non-B subtypes in most provinces, the present results suggest that the proportion of non-B subtypes varies between geographic regions. The CHSDRSP pilot study identified no non-B subtypes from Newfoundland, whereas up to 15.6% of the samples from British Columbia belonged to this group. However, a specimen from an African-born person in Newfoundland was identified as subtype A by the CHSDRSP reference services. Also, the only sample identified as subtype D from the CHSDRSP pilot study was collected from an individual of African origin. Further phylogenetic analysis is required to confirm whether the relatively high proportion of non-B subtypes in British Columbia may be related to a cluster of infections several years ago, as suggested by the majority of non-B subtypes being found in 1996. Cluster investigations similar to the study conducted by Montpetit et al (7) in Newfoundland would also help clarify this matter.

A higher prevalence of non-B subtypes may be directly related to the presence of mobile populations and immigration from countries where divergent HIV subtypes are found. More data collection and analysis are required to confirm this hypothesis, but the impacts of travel and migration on blurring geographic distinctions of subtype distribution are well documented. The deployment of army personnel, for example, has been associated with the introduction of non-B subtypes into the United States (28), Uruguay (29) and France (30). Non-B subtypes have also been identified among immigrant populations originating from HIV-endemic countries living in the United States (27,31), England (22), Sweden (32), Netherlands (33), France (34) and Israel (35).

The introduction of variant HIV subtypes into Canada will invariably challenge existing diagnostic tests and/or interpretation algorithms. Depending on future findings related to the transmissibility, pathogenicity and treatment implications of various subtypes, it may also play a role in changing the nature of the HIV epidemic in Canada. It is, therefore, imperative to implement a systematic collection and analysis of data related to strain surveillance across Canada.

FUTURE DIRECTIONS

Although the use of highly active antiretroviral therapy (HAART) has led to a reduction in HIV-1 related morbidity and mortality in the developed world, HAART has created a unique set of challenges – the development of resistance to antiretroviral drugs that greatly affect the treatment of HIV-1 infection. Much more remains to be learned about the transmission of drug-resistant strains and the implications for the clinical management of individuals infected with antiretroviral resistant HIV.

One of the central goals of CHSDRSP is to monitor drug-resistant strains of HIV, in particular to assess the extent of transmission of drug-resistant HIV-1 variants to drug-naïve individuals. This information may be used to guide clinical management decisions and to develop guidelines for initial therapy at the population level. It may also help enhance coverage for the treatment of newly diagnosed HIV-positive cases in Canada. The epidemiological component of resistance surveillance consists of an existing HIV surveillance database plus additional information collected to help interpret the laboratory information. The laboratory component involves the collection of serum from all new HIV diagnoses for sequencing of polymerase gene *pol* PCR products to determine

mutations associated with resistance to individual or groups of antiretroviral drugs.

We expect that preliminary drug resistance results will be available within a few months, and the CHSDRSP pilot will be expanded to include all provinces and territories by the end of 2000.

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