HIV-1 Subtype Diversity in Minnesota

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(See the editorial commentary by Bennett, on pages 4–9.)

Background. Genetic variation in human immunodeficiency virus (HIV)–1 poses significant public-health and clinical challenges. In North America, subtype B is most prevalent. HIV-1 subtyping is not integrated into routine HIV/acquired immunodeficiency syndrome surveillance in the United States. In 2003, the Minnesota Department of Health piloted HIV-1 subtyping with routine surveillance to describe the existence and variety of non–subtype B strains.

Methods. Targeted HIV-1 subtype surveillance was conducted on 98 African-born HIV-infected patients. Sentinel subtype surveillance was conducted in a Minneapolis sexually transmitted disease clinic on 28 newly diagnosed non-African HIV-positive patients. Subtype determination was based on a partial sequence of the gp41 region of the HIV-1 env gene.

Results. Subtyping was successful for 87 of 98 samples from African-born HIV-infected patients; 95% were non-B subtypes. The 7 subtypes observed were consistent with strains endemic in patients’ birth regions. Subtyping was also completed for samples from 25 of 28 non–African-born patients; all were subtype B.

Conclusions. Multiple HIV-1 subtypes are present in Minnesota. Our data suggest that most of the HIV cases in Minnesota among African-born patients are non-B subtypes. Population-based surveillance inclusive of groups at high risk for variant strains is needed to monitor the prevalence and variety of HIV subtypes in the United States.

The extensive genetic variability of HIV-1 is a significant characteristic of the virus and of the HIV/AIDS pandemic. A subtype nomenclature was developed to describe the variation resulting from mutation and recombination of the viral genome [1]. Of the 3 main HIV-1 subtype groups (major [M], outlier [O], and non-M, non-O [N]), group M predominates in the global epidemic and exhibits the greatest genetic diversity. At present, 11 group M nonrecombinant subtypes and sub-subtypes and 16 circulating recombinant forms (CRFs) are recognized [2].

HIV-1 subtypes are differentially distributed around the world. Whereas subtype B is most prevalent in North America, western Europe, and Australia, subtypes A, C, D, and CRF02_AG predominate in Africa [3, 4]. Novel HIV clades may be quickly introduced to regions via migration, travel, and return from military service abroad [5, 6].

The substantial genetic variation of HIV-1 poses significant public-health and clinical challenges. Specifically, diagnostic testing, patient monitoring, treatment, vaccine development, and epidemiologic surveillance are affected by the genetic diversity of HIV [3, 7]. The relationships between viral subtype and transmissibility, infectivity, and pathogenicity remain uncertain [7–11].

The importance of public-health surveillance for monitoring the frequency and variety of HIV subtypes in the United States [12] was recognized shortly after the nation’s first case of a non–subtype B HIV-1 infection was described in 1994 [13]. Since then, several reports on surveillance for variant HIV strains in special populations (e.g., military personnel, clinical populations, and blood donors), as well as multiple case reports of non–subtype B HIV-1 infections, have appeared [14–31]; however, only 1 population-based surveillance report has been published that included data on persons with HIV infection diagnosed through 31 January 1997 [32].

Because HIV genetic diversity is a significant feature of local, national, and global epidemics and because...
Minnesota is a common US location for immigration by Africans, the Minnesota Department of Health (MDH) enhanced its routine disease-surveillance activities, to describe and monitor HIV-1 subtypes in Minnesota. The ethnic diversity of the HIV/AIDS epidemic in Minnesota has increased dramatically during recent years, primarily because of increasing rates of HIV infection among African immigrants [33]. We assumed that patients with HIV infection who originated from regions where non-B subtypes predominate, such as Africa, would be more likely than US-born patients to be infected with a variant (non-B) strain of HIV.

PATIENTS AND METHODS

Targeted surveillance. Targeted surveillance to describe the existence and variety of HIV-1 subtypes found in Minnesota and sentinel surveillance to monitor for the introduction of non-B subtypes into the native Minnesota HIV epidemic were implemented in early 2003; specimens submitted through 3 March 2004 are included in the present article. This pilot surveillance project was deemed to be nonresearch by the local institutional review board; all patients were informed about the project and were given the opportunity to refuse participation.

A cross-sectional convenience sample of African-born patients who were receiving medical care for HIV infection was identified. We selected 3 HIV clinics (Hennepin County Medical Center [HCMC], Minneapolis; Regions Hospital, St. Paul; and Parkway Clinic, Robbinsdale) in the Minneapolis–St. Paul area that provided HIV care to nearly 60% (unpublished data, Minnesota Department of Health) of the 335 known African-born persons who received a diagnosis of HIV infection through 31 December 2002 in Minnesota [34]. All 3 clinics agreed to participate in targeted HIV-1 subtype surveillance.

Beginning in February 2003, Regions Hospital HIV Clinic and Parkway Clinic patients meeting the following criteria were included:

1. they reported a country of birth other than the United States,
2. they were infected with HIV and were receiving medical care at a participating clinic,
3. they resided in Minnesota at the time of survey (including temporary residents—e.g., students or long-term visitors), and
4. they (or their parent/guardian) agreed to provide a blood sample to the MDH for determination of the HIV subtype.

Beginning in June 2003, HCMC HIV clinic patients meeting the following criteria were included:

1. they reported an African country of birth,
2. they were infected with HIV-1 and were receiving care from a doctor and/or registered nurse at the HCMC HIV clinic,
3. they provided written or oral informed consent,
4. they resided in Minnesota for at least 2 months of the year and were at least 18 years old at the time of survey.

Two 10-mL samples of blood anticoagulated in EDTA that had been collected from consenting patients during routine blood draws were sent to the MDH Public Health Laboratory for molecular subtyping; a unique identifier system was used to maintain patient confidentiality. Generally, within 3 working days of receiving subtype results from the MDH Public Health Laboratory, MDH HIV/AIDS surveillance staff communicated these results to a designated staff member at the submitting clinic.

MDH HIV/AIDS surveillance staff later appended each patient’s subtype results to his or her existing surveillance case report. Surveillance case reports are routinely received from physicians and laboratories, in accordance with Minnesota state rules [35, 36]. Standard data collected included country of birth and other demographic characteristics, as well as information on transmission risk.

Sentinel surveillance. The Red Door Clinic (RDC), a publicly funded sexually transmitted disease clinic that serves a diverse cross-section of the Minneapolis–St. Paul urban and suburban populations, agreed to participate in sentinel HIV-1 subtype surveillance. Subtype determination was attempted on all new Western-blot–positive specimens submitted to the MDH Public Health Laboratory for HIV testing from RDC between January 2003 and March 2004.

HIV-1 subtype characterization. Buffy coat was collected from samples after centrifugation for 5 min at 2800 g; this was stored at −70°C. Proviral DNA was extracted from buffy coat by use of the QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer’s protocol, with 1 modification: samples were heated at 56°C for 30 min, to inactivate viable virus.

An ~400-bp fragment of the gp41 region of the env gene was amplified by use of a nested polymerase chain reaction (PCR), according to the method of Brennan et al. [37], except that proviral DNA rather than cDNA was used as the template. Proviral DNA was used as the PCR target, to allow amplification of virus in samples from patients with low viral loads. PCR products were separated by electrophoresis through an agarose gel and were visualized by use of UV light. Negative and positive controls were included in each reaction, to monitor for potential cross-contamination of samples and to detect problems with PCR conditions and reagents.

Amplified fragments were purified for sequencing by use of a QIAquick PCR Purification Kit (Qiagen), according to the manufacturer’s protocol. The inner primers env27F and env19R were used for automated DNA sequencing by use of a Beckman Coulter CEQ 8000 Genetic Analysis System with the CEQ DTCS Quick Start Kit (Beckman Coulter). The derived nucleotide sequences were aligned and edited by use of Vector NTI Advance (Invitrogen).

For subtype determination, sequences from study-sample
isolates were compared with reference sequences by use of the NCBI Blast genotyping tool [38]. Evidence for recombination was assessed by comparing sequences from patient samples with reference sequences for each of the individual HIV subtypes. Phylogenetic analysis was performed to confirm subtype assignment and to identify potential problems with the cross-contamination of samples. Sequences were aligned, and phylogenetic relationships were determined by the neighbor-joining method by use of the Kimura 2-parameter distance correction. All analyses were performed by use of the sequence and cluster analysis modules of the BioNumerics software platform (version 4.00; Applied Maths). Tree reproducibility was evaluated by bootstrap analysis. The sequence of simian immunodeficiency virus strain CPZANT was used as an outgroup.

**Analytical and statistical methods.** The Minnesota HIV/AIDS surveillance system database was used to compare patient data in the targeted and sentinel surveillance with those from all prevalent patients with HIV infection among African immigrants and all non-African patients who received a diagnosis in 2003, respectively. Data on African-born patients with HIV infection identified through RDC were reassigned to targeted surveillance. The Minnesota HIV/AIDS surveillance system database was used to compare patient characteristics of patients in targeted surveillance with those from all prevalent patients with HIV infection among African immigrants and all non-African patients who received a diagnosis in 2003, respectively. Data on African-born patients with HIV infection identified through RDC were reassigned to targeted surveillance (n = 3). Only data from RDC patients who received a diagnosis on or after 1 January 2003 were included in sentinel surveillance. The z-test statistic was used to compare characteristics of each subtype surveillance population with the appropriate total surveillance population. Analyses were conducted by use of the SAS System for Windows (version 8.02; SAS Institute).

**RESULTS**

**Targeted surveillance.** Specimens from 98 prevalent African-born patients with HIV infection were received between February 2003 and March 2004. Of these patients, 55% (54/98) were women, 44% (43/98) were <35 years old, 80% (78/98) had received a diagnosis with HIV infection in 1998 or later, 87% (85/98) had been born in East or West Africa, and 9% (9/98) had been born in Central Africa (table 1). The majority (79/98 [81%]) of patients had an undetermined mode of exposure, mainly because of challenges faced by MDH disease investigators, whose attempts to interview African patients with new diagnoses are routinely hampered or precluded by language and cultural barriers (the majority of patients are believed to have acquired HIV through heterosexual transmission). PCR amplification was successful for 89% (87/98) of specimens. Twenty-six percent (25/98) of patients received a diagnosis of AIDS at the time of diagnosis of HIV infection. The characteristics of patients in targeted surveillance were similar to those of all prevalent African-born patients reported to the MDH through 31 December 2003 (table 1).

A total of 7 different HIV subtypes were identified through targeted HIV-1 subtype surveillance. Figure 1 shows the results of a phylogenetic tree analysis of the amplifiable gp41 sequences and illustrates the substantial HIV genetic diversity present in Minnesota. Subtypes C, A, and CRF02_AG/A1 (a Blast [38] database search indicated that the closest match was to CRF02_AG; however, recombination analysis indicated that the region sequenced was subtype A1, with no evidence of recombination with subtype G) were most common, constituting 80% (70/87) of the total. Two samples were designated as subtype C by use of the NCBI HIV genotyping tool [38]; however, the sequences were distinct from those of the other subtype C isolates. These 2 isolates are designated on the phenogram in figure 1 as untypeable/C.

Nearly all amplified samples from African-born patients with

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Targeted surveillance (n = 98)</th>
<th>All prevalent African-born patients&lt;sup&gt;a&lt;/sup&gt; (n = 419)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44 (45)</td>
<td>201 (48)</td>
</tr>
<tr>
<td>Female</td>
<td>54 (55)</td>
<td>218 (52)</td>
</tr>
<tr>
<td>Current age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>43 (44)</td>
<td>190 (45)</td>
</tr>
<tr>
<td>≥35 years</td>
<td>55 (56)</td>
<td>229 (55)</td>
</tr>
<tr>
<td>Year of initial HIV diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997 or before</td>
<td>22 (22)</td>
<td>85 (20)</td>
</tr>
<tr>
<td>1998–2004</td>
<td>78 (80)</td>
<td>334 (80)</td>
</tr>
<tr>
<td>Place of birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>East Africa&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67 (68)</td>
<td>252 (60)</td>
</tr>
<tr>
<td>West Africa&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18 (18)</td>
<td>99 (24)</td>
</tr>
<tr>
<td>Central Africa&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9 (9)</td>
<td>35 (8)</td>
</tr>
<tr>
<td>Southern Africa&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4 (4)</td>
<td>33 (8)</td>
</tr>
<tr>
<td>Mode of exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual contact</td>
<td>16 (16)</td>
<td>49 (12)</td>
</tr>
<tr>
<td>Homosexual contact</td>
<td>3 (3)</td>
<td>16 (4)</td>
</tr>
<tr>
<td>Undetermined&lt;sup&gt;f&lt;/sup&gt;</td>
<td>79 (81)</td>
<td>354 (84)</td>
</tr>
<tr>
<td>Status at initial diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infected (non-AIDS)</td>
<td>73 (74)</td>
<td>308 (74)</td>
</tr>
<tr>
<td>AIDS</td>
<td>25 (26)</td>
<td>111 (26)</td>
</tr>
<tr>
<td>PCR amplification of specimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful</td>
<td>87 (89)</td>
<td>…</td>
</tr>
<tr>
<td>Unsuccessful</td>
<td>11 (11)</td>
<td>…</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of subjects. Percentages for place of birth in targeted surveillance add up to 99% because of rounding. There were no statistical differences between targeted subtype surveillance cases and all prevalent African cases for the listed variables at the level by use of the 2-tailed z-test statistic. PCR, polymerase chain reaction.

<sup>a</sup> Source: Minnesota Department of Health HIV/AIDS Surveillance System.

<sup>b</sup> Burundi, Ethiopia, Kenya, Rwanda, Somalia, Sudan, Tanzania, and Uganda.

<sup>c</sup> Côte d’Ivoire, Gambia, Ghana, Guinea, Liberia, Mali, Nigeria, Sierra Leone, and Togo.

<sup>d</sup> Cameroon, Congo, and Democratic Republic of Congo (formerly Zaire).

<sup>e</sup> Botswana, Malawi, South Africa, Zambia, and Zimbabwe.

<sup>f</sup> Unable to be interviewed or insufficient risk information provided to ascertain the mode of exposure.
**Figure 1.** Phylogenetic analysis of the gp41 env gene sequences of targeted surveillance samples. The phenogram was generated as described in Patients and Methods. Nos. at the nodes indicate bootstrap values. Only bootstrap values ≥60% are included. Branches with asterisks represent reference sequences. GenBank accession nos. of reference sequences are as follows: U51188, AF004885, AF069670, AF286238, AF286241, AF286237, K03455, U63632, U52953, AF110967, K03454, M27323, AF077336, AF005494, AJ249236, AJ249237, AF084936, AF061641, AF190127, AF190128, AF082394, AF082395, AJ249235, AJ249239, AF197340, U51188, AJ286133, and AF063224. The sequence of simian immunodeficiency virus strain CPZANT was used to root the tree. U/C, untypeable/type C.

HIV-1 infection in the targeted surveillance were non-B subtypes (83/87 [95%]; table 2). The HIV-1 subtypes observed were generally reflective of endemic strains present in patients' regions of birth [3, 4]. Nearly all patients (58/65 [90%]) with HIV-1 subtype A, D, or C were born in East Africa, whereas subtypes AG and G were observed only among patients born in West or Central Africa. Similarly, 3 of 4 patients born in southern Africa were infected with HIV-1 subtype C.

**Sentinel surveillance.** Between January 2003 and March 2004, 28 specimens were received by MDH, representing 100% of newly diagnosed cases in non-African patients at RDC. Of these patients, 7% (2/28) were women, 50% (14/28) were <35 years old, 64% (18/28) were white, 18% (5/28) were African American, and 18% (5/28) were of other races (table 3). Most patients (21/28 [75%]) had been infected through male-to-male sexual contact. No patient received a diagnosis of AIDS at the time of diagnosis of HIV infection. When patients in sentinel surveillance were compared with all non-African patients who received a diagnosis in Minnesota during 2003, patients in sentinel surveillance were more likely to be male, to have been exposed to HIV through male-to-male sex, and to be AIDS free at the time of diagnosis of HIV infection (table 3).

Eighty-nine percent (25/28) of the specimens were sequenced, and all were characterized as HIV-1 subtype B. Phylogenetic analysis of the gp41 sequences included in sentinel HIV-1 subtype surveillance is presented in figure 2, to illustrate the level of genetic diversity present among presumably circulating subtype B viruses in Minnesota.

**HIV/AIDS among Africans in Minnesota.** Figure 3 depicts the marked increase in diagnoses of HIV infection in the African-born population in Minnesota that began in the mid-1990s. By 2003, 21% (55/266) of patients who received a diagnosis of HIV infection annually in Minnesota were African born.

**DISCUSSION**

In 2003, Minnesota began pilot testing the addition of HIV-1 subtyping to routine HIV/AIDS disease surveillance, to (1) describe the existence and variety of non–subtype B viruses and (2) monitor the indigenous HIV epidemic for the appearance of such viruses. This pilot system was composed of targeted and sentinel surveillance. Targeted surveillance data demonstrated that multiple HIV-1 subtypes are present in Minnesota and that they reflect strains that are prevalent in patients' regions of birth. Although no variant strains of HIV-1 were detected among the non-African patients at RDC, we are not confident about generalizing these findings to the entire indigenous epidemic in Minnesota, because the number of patients in sentinel surveillance represented only 13% (28/211) of Minnesota’s indigenous patients who received diagnoses in 2003 and underrepresented women and heterosexually transmitted infections. Our findings do demonstrate that, in the population...
served by this clinic, non-B HIV-1 subtypes seem to be limited to patients born outside of the United States.

A similar pattern of the introduction and establishment of novel HIV-1 subtypes has been observed in many western European countries, particularly those with less restrictive immigration policies (in contrast to countries such as England and France, the United States does not generally permit persons with HIV infection to immigrate) and/or former colonies in sub-Saharan Africa [39–53]. For example, after the first description in 1995 of non-B HIV-1 subtypes in the United Kingdom among persons with epidemiologic links to sub-Saharan Africa [54], a national effort to monitor the genetic diversity of the epidemic was proposed in 1996 [55]. Subsequent reports documented considerable variation and a high prevalence of non–subtype B HIV-1 strains; in 1998, non-B subtypes were estimated to account for 27% of prevalent HIV infections in the United Kingdom, were not limited to infections acquired abroad or to particular immigrant populations, and were almost exclusively associated with heterosexual exposure [39, 56]. Of all reported HIV infections in the United Kingdom diagnosed through the end of 2001, 21% (9993/48,226) were probably acquired in Africa [57].

Similar to the experience in other locations, the primary sources of non–subtype B HIV-1 infections in Minnesota are believed to be migration from and travel to areas where HIV is highly prevalent and non-B subtypes predominate [57, 58]. Specifically, extensive immigration from African countries to Minnesota occurred between 1996 and 2003, during which period >13,000 African immigrants resettled in Minnesota [59, 60]. This does not include the unknown number of secondary refugees/immigrants, persons holding various work or student visas, undocumented immigrants, or visitors. The term “secondary refugees/immigrants” refers to persons who initially arrived in other parts of the United States as refugees or other immigrants and who subsequently moved to Minnesota. No formal documentation of these movements exists, so quantifying the arrivals of secondary refugees/immigrants in Minnesota is impossible. According to 2000 US Census data, Minnesota is home to the tenth largest African population in the United States and the second largest East African population [61]. Many of these individuals travel home to visit family and friends remaining in Africa.

The dramatic increase in the prevalence of HIV infections among African-born persons in Minnesota during 1996–2003 is only partly explained by the concomitant increase in population. Despite making up <1% of the state’s general population [61], Africans accounted for 4% (12/292) of all newly diagnosed HIV infections in 1996 and for 21% (65/305) by 2002 [34]. Furthermore, the large percentage (95%) of non-B

Table 2. HIV-1 subtype, by African region of birth, among the 87 patients in targeted surveillance with typeable virus in Minnesota, February 2003–March 2004.

<table>
<thead>
<tr>
<th>Region of birth</th>
<th>B</th>
<th>A1</th>
<th>C</th>
<th>D</th>
<th>G</th>
<th>AG</th>
<th>U/C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Africaa</td>
<td>1</td>
<td>17</td>
<td>33</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>West Africab</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Central Africac</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Southern Africad</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>21</td>
<td>36</td>
<td>8</td>
<td>4</td>
<td>13</td>
<td>1</td>
<td>83</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of patients. U/C, untypeable/type C.

Table 3. Characteristics of patients in sentinel HIV-1 subtype surveillance and of all non-African patients with diagnoses in Minnesota during 2003.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sentinel surveillance (n = 28)</th>
<th>All non-African patients with diagnoses during 2003a (n = 211)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>21 (75)</td>
<td>115 (55)b</td>
</tr>
<tr>
<td>IDU</td>
<td>3 (11)</td>
<td>23 (11)</td>
</tr>
<tr>
<td>Heterosexual contact</td>
<td>2 (7)</td>
<td>18 (9)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>2 (7)</td>
<td>55 (26)b</td>
</tr>
<tr>
<td>Status at initial diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infection (non-AIDS)</td>
<td>28 (100)</td>
<td>145 (69)b</td>
</tr>
<tr>
<td>AIDS</td>
<td>0</td>
<td>66 (31)</td>
</tr>
<tr>
<td>HIV-1 subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>25 (89)</td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>3 (11)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>28 (100)</td>
<td>211 (100)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of subjects. “Undetermined” includes patients who were not interviewed by Minnesota Department of Health (MDH) staff; those whose exposure history was incomplete because they died, declined to be interviewed, or were lost to follow-up; and those who were interviewed or for whom follow-up information was available but no exposure was identified or acknowledged. IDU, injection drug user; MSM, men who have sex with men; NT, unsuccessful amplification of gp41 sequence. MSM/IDU men were included in the IDU category.

a Source: MDH HIV/AIDS Surveillance System.
b Difference between sentinel subtype surveillance patients and all non-African subjects statistically significant at the level by use of the 2-tailed z-test statistic.

Hispanic, Asian, American Indian, or multiple races.
subtypes among patients in targeted surveillance suggests that most African-born patients with HIV infection diagnosed in Minnesota during 2003 (n = 55) were also likely to have non-B subtypes. Consistent with this, only 1 (5%) of 22 patients in targeted surveillance who received a diagnosis during 2003 were infected with a subtype B virus.

Because the majority of African immigration to the United States, including to Minnesota, has occurred relatively recently, cultural assimilation is still minimal. Our data indicate that <5% of HIV-1 infections among Africans are subtype B and that non-B subtypes in the indigenous population are rare, which is consistent with a limited crossover of sexual networks between the 2 populations. However, as has occurred in the United Kingdom [39], we expect that non-B subtypes will appear in the indigenous US HIV epidemic, initially among heterosexual persons, reflecting the primary mode of person-to-person transmission in Africa [62].

Since the first documentation of a non-subtype B HIV-1 infection in the United States [13] and a call for public-health surveillance to track HIV variants in 1996 [12, 6], 6 reports and 8 abstracts on monitoring for or estimating the prevalence of non-subtype B HIV-1 strains in a variety of populations have been published [16, 18–21, 23, 27–32]. Pau et al. [16] reported no cases of group O infection among 1072 serum samples stored between 1987 and 1994 from a variety of groups, including blood donors and military personnel at several sites around the United States and Puerto Rico. De Oliveira et al. [21] concluded that <1% of HIV-seropositive blood donors through 1996 were infected with non-B subtypes.

In a hospital-based cross-sectional study conducted in New York City (NYC) during 1992–1994, 4.7% (2/43) of newly identified HIV-infected patients were infected with HIV-1 subtype A [18]. Of note, only persons who could speak English or Spanish were eligible to participate, which may have limited the ability to detect additional non-B subtypes in the patient population, if present. Sullivan et al. [32] conducted a cross-sectional investigation of African-born, HIV-infected persons reported to the Centers for Disease Control and Prevention’s national population-based surveillance registry as of 31 January 1997 and who were at high risk for group O or N infection on the basis of country of birth; 2 group O and 0 group N infections were identified among 32 typeable specimens. Nearly all participants were born in West or Central Africa, and a large percentage (84% [27/32]) were infected with non-B subtypes.

Renzullo et al. [20] presented data demonstrating a low prevalence (1.7%) of non-subtype B HIV-1 serotypes among 1966 civilian military applicants who tested positive for HIV antibody during 1989–1998. During 1997–1998, a cross-sectional survey of US military personnel stationed in the western United States or the Pacific region overseas and who had recently received a diagnosis of HIV infection found subtype CRF01_AE to account for 8% (7/95) of infections; 6 of 7 cases were epidemiologically linked to Thailand [19].

The NYC Department of Health Retrovirology Laboratory conducts HIV testing for publicly funded health and hospital facilities in NYC; they also accept specimens from patients suspected to be at risk for HIV-2 or variant subtypes of HIV-1 from any New York state licensed physician [63]. Beatrice et al. [26, 27] and Lin et al. [28] have demonstrated large numbers of non-B HIV-1 subtypes in the foreign-born population of NYC. Between 1993 and 1998, they found that 68% (351/517) of specimens from African-born persons were reactive to non–subtype B HIV-1 peptides (non-B serotype); the percentages among Asian-born and South American–born persons were 51% (26/51) and 41% (83/201), respectively [27]. There are data to suggest that the percentage of non-B subtypes among Africans in NYC may actually be higher. Kahirimbanyi and Brutsaert [29] reported in an abstract that >90% of 83 African
Figure 3. Annual nos. of HIV infections (HIV or AIDS at first diagnosis) diagnosed among African-born persons residing in Minnesota, by year of diagnosis, 1990–2003.

and Caribbean patients who tested positive for HIV through an NYC community-based African services organization between 1999 and 2001 had a non-B HIV-1 subtype.

HIV-1 subtyping based on protease and reverse-transcriptase sequences from 2246 patients in northern California for whom drug-resistance testing was ordered during 1997–2000 indicated that <1% were infected with non-B subtypes [23]. Another study analyzed 5322 specimens submitted for drug-resistance genotyping during the previous year and found only 8 (0.15%) non-B subtypes on the basis of sequencing the pol region [64].

Immigrant populations, including those from regions where variant HIV-1 subtypes are endemic, often face significant barriers to accessing HIV medical care, including language, stigma, distrust of government, and a lack of resources [65] (E. Namarra, MDH African HIV/AIDS Project, personal communication). Consistent with the general process of cultural assimilation, Africans who arrived in the United States longer ago are more likely than recent immigrants to have sufficient knowledge and trust of the US health-care system to access testing and care and to have had contact with domestic sexual networks (where HIV-1 subtype B predominates). Therefore, prevalence estimates based solely on specimens from health-care facilities or sequences available from drug-resistance monitoring are likely to underestimate the true prevalence in the population.

Similarly, the US prevalence estimates of non-B subtypes reported by Zaidi et al. at conferences in 2000 and 2002 (1.7% and 2%, respectively) [30, 31] were not derived from population-based samples but from samples from persons in 10 US cities who had been willing to enroll in a government-run study and be tested for HIV. Thus, the resulting estimates are likely to be low, given that the US residents most likely to have non-subtype B infections—that is, foreign-born persons—are also the most likely to decline participation in such a study [66].

Because most studies of antiretroviral therapy (ART) and resistance have involved subjects from resource-rich regions of the world where HIV-1 subtype B is the predominant strain, data regarding non-subtype B strains are still limited. There are some data to suggest that disease-progression rates may differ by subtype [9–11, 67]. Similarly, limited data have suggested that there may be clinically important differences in drug-resistance pathways and/or coreceptor use between HIV subtypes [68–76]. We (K.H. and O.A.) have recently treated 3 African-born patients with AIDS who were naive to ART and were infected with non–subtype B HIV-1 that was found to have the K103N mutation on baseline genotyping [77], which underscores the clinical importance of this issue. Key issues pertaining to HIV-1 subtypes include diagnosis of infection, quantification of viral loads, drug-resistance pathways, response to ART, challenges to vaccine development, and transmissibility; these highlight the importance of tracking viral diversity in a variety of settings.

To our knowledge, this is the first report of incorporating HIV-1 subtype determination into routine public-health HIV/AIDS case surveillance to monitor the diversity of HIV-1 subtypes present in the United States. The high percentage of variant HIV-1 strains found among representative African patients in Minnesota, coupled with continued high levels of travel and immigration from Africa and other areas, such as southeast Asia, where non-B HIV-1 subtypes predominate, confirm the
need for effective surveillance to monitor the prevalence and distribution of HIV variants in the United States.

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