

The lack of association between bacille Calmette-Guérin vaccination and clustering of Aboriginals with tuberculosis in western Canada

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BACKGROUND: Tuberculosis (TB) remains a major health problem for Aboriginal people in Canada, with high rates of clustering of active TB cases. Bacille Calmette-Guérin (BCG) vaccination has been used as a preventive measure against TB in this high-risk population.

OBJECTIVES: The study was designed to determine if BCG vaccination in Aboriginal people influenced recent TB transmission through an analysis of the clustering of TB cases.

METHODS: A retrospective analysis of all culture-positive *Mycobacterium tuberculosis* cases in Aboriginal people in western Canada (1995 to 1997) was performed. Isolates were analyzed using standard methodology for restriction fragment length polymorphism and spoligotyping.

RESULTS: Of 256 culture-positive Aboriginal TB cases, BCG status was confirmed in 216 (84%) cases; 34% had been vaccinated with BCG, 57% were male and 56% were living on-reserve. Patients who had been vaccinated with BCG were younger than unvaccinated individuals (mean age 32.4 ± 1.65 years versus 45.0 ± 1.8 years, $P < 0.0001$). Clustering was found in 62% of cases: 59% of non-BCG vaccinated cases were clustered versus 68% of those vaccinated with BCG ($P = 0.16$). Younger patients (younger than 60 years of age) were more likely to be clustered in the univariate analysis ($P < 0.01$). When age, sex, province, and HIV and reserve status were controlled for, BCG vaccination was not associated with clustering (OR 1.3, 95% CI 0.7 to 2.6).

CONCLUSIONS: BCG vaccinated Aboriginal people were no less likely to have active TB from recently transmitted disease. BCG vaccination appears to have limited value in preventing clustering of TB cases within this high-risk community.

Key Words: BCG vaccination; Clustering; Molecular epidemiology; Tuberculosis

Tuberculosis (TB) remains a major public health problem for Aboriginal people in Canada (1). Although rates of TB in Aboriginal Canadians continue to fall, the incidence of TB is still more than 10 to 15 times that of the non-Aboriginal Canadian population (2). The principal means of controlling TB in Aboriginal communities follow national guidelines:

Absence de lien entre le vaccin BCG et les cas de tuberculose chez des Autochtones de l'Ouest canadien

HISTORIQUE : La tuberculose (TB) reste un important problème de santé publique chez les Premières nations du Canada, avec des taux élevés de TB active. Le vaccin BCG (bacille de Calmette-Guérin) a été utilisé comme mesure préventive contre la TB auprès de cette population à haut risque.

OBJECTIF : L'étude a été conçue afin de déterminer par le biais d'une analyse typologique des cas de TB quel a été l'effet d'un vaccin BCG sur les Premières nations lors d'une propagation récente de la maladie.

MÉTHODES : Analyse rétrospective de tous les cas de *Mycobacterium tuberculosis* positifs à la culture chez les Premières nations de l'Ouest canadien (1995 à 1997). Les isolats ont été analysés à l'aide d'une méthodologie standard de polymorphisme de restriction et de spoligotypage.

RÉSULTATS : Parmi 256 cas de TB positifs à la culture chez les Autochtones, le statut à l'égard du BCG a été confirmé dans 216 cas (84 %); 34 % avaient reçu un vaccin BCG, 57 % étaient des hommes et 56 % vivaient sur une réserve. Les patients qui avaient été vaccinés avec le BCG étaient plus jeunes que les individus non vaccinés (âge moyen $32,4 \pm 1,65$ ans vs $45,0 \pm 1,8$ ans; $P < 0,0001$). Des concentrations de cas ont été retrouvées dans une proportion de 62 % : 59 % des cas non vaccinés par le BCG ont été comparés aux 68 % des sujets vaccinés ($P = 0,16$). Les patients plus jeunes (de moins de 60 ans) étaient plus susceptibles de faire partie des concentrations de cas lors de l'analyse univariée ($P < 0,01$). Lorsque l'on tenait compte de l'âge, du sexe, de la province de résidence, du VIH et du fait de vivre ou non sur une réserve, le vaccin BCG n'a été associé à aucune concentration de cas (RR 1,3; IC 95 %, 0,7 à 2,6).

CONCLUSIONS : Les personnes autochtones vaccinées par le BCG n'étaient pas moins susceptibles de souffrir d'une TB active récemment transmise. Le BCG semble avoir une utilité limitée pour ce qui est de prévenir la TB au sein de cette communauté à haut risque.

finding active cases and the early initiation of treatment, complete contact investigation and treatment of latent infection, and appropriate infection control measures (3). Bacille Calmette-Guérin (BCG) vaccination has been recommended as an effective preventive measure in this high-risk population for over 40 years (4-6).

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At present, BCG vaccination in Canada is offered to specific high-risk communities, primarily Aboriginal newborns living on-reserve (3). However, the role of BCG vaccination in Canada remains controversial and under review due to questions of efficacy and side effects, which vary from local adverse reactions to disseminated BCG infection (7-12). If it is assumed that BCG vaccination has a protective effect against TB, it is reasonable to suspect that there may be an influence on recent TB transmission in a BCG-vaccinated population because of a reduced chance of disease acquisition.

Molecular epidemiology techniques provide a surrogate marker for recent transmission. TB isolates from culture-positive cases can be fingerprinted using restriction fragment length polymorphism (RFLP) to assess the relatedness. Clustered strains with identical fingerprint patterns suggest recent or ongoing transmission within a community, while unique strains suggest reactivation of the disease or acquisition elsewhere (13,14). The proportion of clustering or recent transmission within Aboriginal communities has been estimated to be 50% to 60% from previous studies (14,15). The objective of the present study was to determine if BCG vaccination in Aboriginal people from western Canada influenced TB transmission through an analysis of the proportion of TB cases that were clustered.

METHODS

Study design

The present study involved the analysis of data collected prospectively for the Canadian Molecular Epidemiology of Tuberculosis Study Group, an evaluation of all *Mycobacterium tuberculosis*-positive cultures in western Canada over a two-year period from February 1, 1995, to January 31, 1997 (14). Isolates from British Columbia, Alberta, Saskatchewan, Manitoba and a small Aboriginal community in northwestern Ontario (TB control coordinated through Manitoba) were included in the study. A descriptive analysis was performed on the demographic and clinical data on each case, as well as the laboratory evaluation of isolates using standardized molecular techniques to assess recent transmission by clustering.

Patients

All cases of *M. tuberculosis* culture-positive TB in Aboriginal people, on- and off-reserve, were included for analysis. The term Aboriginal is a collective name used to describe the indigenous peoples of North America and their descendants. Aboriginal Canadians include status and nonstatus First Nations people (previously known as Indian), Inuit (of Arctic Canada) and Métis (mixed First Nations and European heritage) as reported by the First Nations and Inuit Health Branch of Canada. Status First Nations people are registered with and recognized by the Canadian government, while nonstatus First Nations people are those who consider themselves members of a First Nation without official recognition by the Canadian government. A reserve has been defined as a tract of land set apart for use and benefit by Aboriginal people. Canadian-born/non-Aboriginal persons who do not routinely receive BCG vaccination and the foreign-born in whom a documented BCG vaccination history is often difficult to confirm were excluded.

Laboratory evaluation

Mycobacterium species were identified using conventional biochemical testing and RNA hybridization probes (16). In western Canada, all cultures for *M. tuberculosis* are carried out in provincial reference laboratories. All positive cultures were sent to the study

laboratory in Edmonton, Alberta, for analysis using molecular epidemiology techniques. Isolates were analyzed using standard methodology and RFLP fingerprinting (described in detail elsewhere [14,15,17]). Spoligotyping (a secondary typing system) was used to discriminate between strains of *M. tuberculosis* with less than six bands (copies) of the IS6110 genotype sequence (18). Standard methods were used to exclude false positive results from laboratory cross-contamination (19). A cluster was defined as two or more isolates within the study group with an identical RFLP fingerprint (regardless of province of diagnosis), while nonclustered cases had a unique RFLP fingerprint. All cases enrolled in the present study were culture-positive and had DNA fingerprinting completed.

Epidemiology evaluation

Intradermal BCG vaccination (Connaught attenuated substrain of *Mycobacterium bovis* BCG; Aventis Pasteur, Canada) is provided, on a voluntary basis, to Aboriginal newborns living on-reserve through the First Nations and Inuit Health Branch of Health Canada (3). The strain and manufacturer of the BCG vaccine was the same for all provinces. Although BCG vaccination is a government policy, coverage is variable because it is left to the discretion of the individual and health care worker. Coverage varies by province and although participation is waning, approximately 50% of all eligible Aboriginal persons have received the vaccine (20). All provinces involved in the study have centralized TB registries where BCG vaccination history is documented. Each registry was contacted for the collection of epidemiological data, including demographic and clinical information for all Aboriginal cases of TB. Demographic information included age at diagnosis, sex and province where the diagnosis was made. Clinical information included history of BCG (yes or no) and HIV infection status. Complete information was collected on predesigned standardized data collection sheets and then entered onto a computer spreadsheet for data analysis.

Statistical analysis

Descriptive statistics of demographic and clinical characteristics were computed using the SAS statistical package (version 8.0, SAS Inc, USA). Associations were evaluated by Student's *t* test, χ^2 and multivariate logistic regression.

RESULTS

During the study period, a total of 944 cases of bacteriologically proven active TB (*M. tuberculosis*) were diagnosed in the four provinces involved in the study. Among Aboriginal people, a total of 256 cases of culture-positive TB in western Canada from February 1, 1995, to January 31, 1997, were identified. Of these 256 cases, BCG status was confirmed in 216 of the total cases (84%). This involved contacting each provincial TB program registry to review TB control and clinical records for formal BCG vaccination information. The 16% of culture-positive Aboriginal TB cases that did not have documentation of BCG status were, therefore, excluded from the analysis. The remaining 216 cases that were analyzed as the study group had confirmed positive or negative BCG vaccination status based on formal documentation for all provinces.

A history of BCG vaccination was documented in 73 (34%) patients (Table 1). Of all cases, 57% were male and 56% were living on-reserve at the time of their TB diagnosis. Patients with a positive history of BCG vaccination were younger than those with a negative history (mean age 32.4 \pm 1.65 years versus 45.0 \pm 1.8 years, respectively; *P*<0.0001).

TABLE 1
Patient demographics: No bacille Calmette-Guérin (BCG) vaccination versus BCG vaccination, n (%)

	No BCG, n=143 (66)	BCG, n=73 (34)	Total, n=216 (100)	P*
Sex†				
Male	81 (66)	41 (34)	122 (100)	0.92
Female	61 (67)	30 (33)	91 (100)	
Age at diagnosis (years)				
0–25	31 (62)	19 (38)	50 (100)	0.0007
26–60	76 (60)	51 (40)	127 (100)	
60+	36 (92)	3 (8)	39 (100)	
Province of diagnosis				
Province A	40 (77)	12 (23)	52 (100)	0.35
Province B	12 (57)	9 (43)	21 (100)	
Province C	33 (65)	18 (35)	51 (100)	
Province D	58 (63)	34 (37)	92 (100)	
Reserve status				
On-reserve	75 (63)	45 (37)	120 (100)	0.19
Clustered cases	84 (63)	50 (37)	134 (100)	0.16

*Using χ^2 analysis; †Sex total is n=213 because three missing values were not available from the database

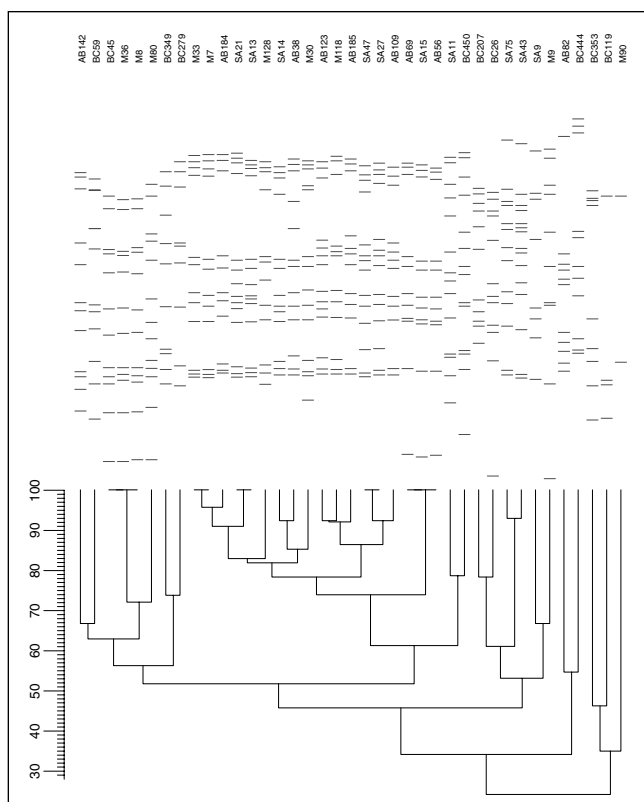


Figure 1) A representative sample (randomly selected) of Aboriginal isolate DNA fingerprints

Of the 216 culture-positive cases of TB in Aboriginal Canadians, only 12 isolates had fewer than six bands (low-copy) and went onto spoligotyping. Clustering was found in 62% of cases of culture-positive TB in Aboriginal Canadians (Table 1 and Figure 1). Of the 12 low-copy strains, five (42%) were clustered by spoligotyping compared with 63% of the high-copy strains clustered by RFLP ($P=0.13$). Clustering occurred in 59%

TABLE 2
Multivariate analysis of clustering of Aboriginal tuberculosis cases*

	OR	95% CI
Age <25 years	2.7	0.9 to 7.1
Age 26 to 60 years	2.1	0.9 to 4.8
Female sex	0.5	0.3 to 0.9
BCG vaccination	1.3	0.7 to 2.6
HIV-positive	1.4	0.4 to 4.7
Off-reserve	1.3	0.7 to 2.5
Province A	0.2	0.1 to 0.6
Province B	0.1	0.03 to 0.3
Province D	0.3	0.1 to 0.8

*Reference groups older than 60 years, Province C (elderly defined as older than 60 years). BCG Bacille Calmette-Guérin

TABLE 3
Province of diagnosis and clustering of tuberculosis cases, n (%)

	Clustered n=134 (62)	Nonclustered n=82 (38)	Total n=216 (100)	P*
Province A	29 (56)	23 (44)	52 (100)	0.29
Province B	8 (38)	13 (62)	21 (100)	0.02
Province C	42 (82)	9 (18)	51 (100)	0.0006
Province D	55 (60)	37 (40)	92 (100)	0.56

*Using χ^2 analysis; each province is compared with all other provinces combined

of non-BCG cases compared with 68% of BCG cases, and this difference was not statistically significant ($P=0.16$). Age was found to be the most significant predictor for clustering in the univariate analysis because younger patients were more likely to be clustered ($P<0.01$). After controlling for age, sex, province, and HIV and reserve status, BCG vaccination was not associated with clustering by logistic regression (OR 1.3, 95% CI 0.7 to 2.6) (Table 2).

No significant difference was observed in BCG vaccination status among the provinces despite the variable coverage (Table 1), although the province of diagnosis appeared to influence clustering (Table 3). Province B had the lowest proportion of clustered cases (38%), while Province C had the highest proportion of clustered cases (82%). When looking at BCG-vaccinated patients alone ($n=73$), no relationship was observed between the province of diagnosis and clustering (data not shown). It was also determined that BCG vaccination was not associated with cluster size or whether the patient was considered to be the source case of the cluster or secondary case by date of diagnosis (data not shown).

A subgroup analysis was performed on the cases of HIV/TB coinfection in the study population. Clustering was found in 69% of these cases (11 of 16 cases): 58% of non-BCG (seven of 12 cases) versus 100% of BCG cases (four of four cases) ($P=0.17$, Fisher's exact test). HIV status was not associated with clustering in the multivariate analysis (OR 1.4, 95% CI 0.4 to 4.7, Table 2).

DISCUSSION

The role of BCG vaccination in Canada remains controversial, although it is generally agreed that vaccination in infancy provides protection against the most severe forms of TB (8,9,21). Although the more severe and reportable adverse

reactions to BCG vaccination remain uncommon, disseminated BCG infection and death in patients with previously unrecognized congenital and acquired immune deficiencies have been reported (11,12). The reduction in TB incidence and the concern regarding BCG-associated deaths has fuelled the debate on BCG vaccination in Canada.

The present study was designed to assess the influence of BCG vaccination on the clustering of TB cases in the Aboriginal communities of western Canada. The administration of BCG to an unvaccinated individual is thought to enhance cell-mediated immunity in the face of subsequent exposure to the tubercle bacillus. Rapid mobilization of host immunity to prevent dissemination is understood to be the mechanism that reduces the risk of miliary and meningeal TB, an effect of great clinical significance in the pediatric population. The BCG vaccine's ability to prevent TB infection in an older population is less efficacious but may limit the progression of infection to disease in approximately 50% of those vaccinated (8,9). If the BCG vaccine can influence the development of disease at the time of initial infection, it would be reasonable to assume that a BCG-vaccinated person would less likely be involved in active transmission due to their intrinsic reduced chance for disease. Therefore, a BCG-vaccinated person would less likely be involved in a clustering of TB cases as a marker of ongoing disease transmission. However, this association was not found in our study population. BCG-vaccinated Aboriginal people were no less likely to have active TB from recently transmitted disease than were unvaccinated individuals.

Aboriginal communities are an important population in which to study TB transmission because the incidence of TB is much higher than the Canadian average, and in recent studies (14,15), being an Aboriginal person was a strong predictor of clustering of TB cases. These results, as well as the present study's reported rate of clustering (63%), confirm ongoing transmission in Aboriginal communities (Table 1). Explanations for the higher incidence of TB among Aboriginal communities have included the remoteness of communities, health worker shortages, housing and socioeconomic conditions, substance abuse, the prevalence of latent TB infection and, more recently, the impact of HIV coinfection (1,20,22,23).

The investigation of DNA fingerprints in western Canada has confirmed the heterogeneity of clusters across ethnic and provincial boundaries (14). In the present study, only 24% (32 of 134) of Aboriginal persons were clustered with other Aboriginal persons only (data not shown). This genotypic diversity is especially relevant to Aboriginal persons, the focus of the present study, in that a unique strain may be interpreted as endemic, leading to the potential overestimation of clustering. Studies (24,25) have suggested that large clusters of TB cases may represent historic transmission of an endemic or unique strain in both Aboriginal and non-Aboriginal Canadian populations. The identification of mixed clusters in the present study confirms that clustering within the Aboriginal communities of western Canada is not solely driven by unique strains within this ethnic group. The concurrent use of multiple genotyping methods, including mycobacterial interspersed repetitive units, has been suggested to provide a more conservative estimate of clustering (25).

We determined that younger patients were more likely to be in a cluster (Table 2). Young people are an important population in which to assess the impact of BCG vaccination on

TB transmission because BCG vaccination does not provide absolute or permanent protection into adulthood. If given in infancy, the protective effect of BCG vaccination may, at best, last 15 to 20 years postinoculation, although there is no agreement on the duration of immunity and efficacy (26-28). While clustering is more common in the young, older patients can develop active disease from reactivation of a latent infection and then become a potential source of disease transmission (13-15). Due to the time-sensitive efficacy of BCG vaccination, it may not have been reasonable to expect vaccination status to influence the rates of clustering found in older Aboriginal people in the present study.

The current study established that younger Aboriginals were more likely to be involved in clusters irrespective of BCG status. Even when controlling for age, sex, province, and HIV and reserve status, there was no significant difference in clustering based on BCG vaccination status. When patients younger than 26 years of age were analyzed, in whom BCG vaccination might be expected to have the strongest influence, we found no significant difference with respect to BCG vaccination status and clustering (data not shown). We also examined the possibility that BCG vaccination influenced the number of large cluster outbreaks, although we found no association between BCG vaccination status and the size of clusters. Although BCG vaccination might provide some protection against the development of TB, there does not appear to be any influence on recent TB transmission through cluster analysis within this Aboriginal cohort.

During the study period, the incidence of TB in Aboriginals varied by province based on population statistics from Health Canada (2). Although the proportion of clustered cases varied by region, with the majority of clustering occurring in the provinces with the highest incidences, the extent of BCG coverage did not appear to have any influence on TB transmission in the present cohort. It should be noted that Province C, with the highest incidence of TB and the highest rate of clustering in the current study, has had one of the highest burdens of TB disease in Canada for the past decade. In 2001 alone, the incidence of TB in Aboriginals in Province C was more than three times the incidence of all Aboriginals in Canada (2). A subanalysis of Aboriginal TB cases in Province C showed no association between clustering and BCG vaccination (data not shown).

HIV infection is a well-documented risk factor for the development of TB and the clustering of cases (29). While the official rate of HIV/TB coinfection in Canada is not yet available, the impact of HIV on TB transmission and clustering during our study period was likely minimal due to the low prevalence of coinfection (29). In those patients with confirmed HIV/TB coinfection ($n=16$), we were unable to detect a difference in clustering by BCG vaccination which might have been expected given the potential for significant immune suppression. One could argue that any effect of BCG vaccination would be lost in HIV-positive patients that are severely immune compromised. However, with HIV-positive patients excluded from the analysis, we were still unable to find a difference with respect to BCG vaccination status and clustering. It remains inherently difficult to assess the contribution of HIV infection to the high rate of clustering in the present study population. Given the increased risk of HIV infection among high-risk Aboriginal communities, it is possible that the potential impact of HIV on TB transmission in this community has yet to be determined (22).

Cluster outbreaks are a major challenge confronting all TB control programs. Aboriginal people who received BCG vaccination were no less likely to have active TB from recently transmitted disease. Therefore, BCG vaccination seems to have limited value in preventing the clustering of TB cases within this high TB prevalence community. The lack of influence of BCG vaccination on clustering in Aboriginal communities supports the belief that more appropriate strategies may be required to control TB transmission in this high-risk community.

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