ORIGINAL ARTICLES

The Importance of Genotyping of Strains for the Evaluation and Interpretation of 5 School-Based Epidemic Outbreaks of Tuberculosis

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OBJECTIVE: The aim of this study was to describe 5 microepidemics of tuberculosis occurring in schools, establish the risk factors associated with the outbreaks, assess how well a concentric circles strategy for contact tracing predicts infection, and assess the usefulness of genotyping strains in the analysis of the outbreaks.

MATERIAL AND METHODS: The study assessed 5 epidemic outbreaks of tuberculosis using a standard contact tracing procedure. The outbreaks occurred in 2 day nurseries and 2 high schools between 1998 and 2005. Contacts were stratified using a concentric circle system based on level of exposure. DNA fingerprints of the available strains were determined based on the restriction fragment length polymorphism (RFLP) IS6110 and compared with the contact study to interpret the transmission of the infection.

RESULTS: We analyzed 5 outbreaks. Eighty-five contacts were analyzed in the first outbreak, 519 in the second, 116 in the third, 655 in the fourth, and 102 in the fifth. The rate of infection was 31%, 29%, 66%, 37.6%, and 32%, respectively. Secondary cases of active disease were detected: 9 in the first outbreak, 16 in the second, 5 in the third, 6 in the fourth, and 13 in the fifth. RFLP analysis revealed that a single strain was involved in 3 of the outbreaks, and in a fourth, at least 2 strains were involved. In outbreaks 2, 3, and 5, there was a significant association between the degree of contact and the probability of infection ($P<.05$). In all of the outbreaks, the relative risk of developing the disease was associated with the level of exposure.

CONCLUSIONS: Analysis of contacts based on concentric circles of risk predicts the likelihood of infection. RFLP facilitates analysis of complex transmission routes that are not detected using traditional methods of contact screening.

Key words: *Tuberculosis. Microepidemic. Outbreak. School. Restriction fragment length polymorphism.*

Microepidemias de tuberculosis en 5 brotes escolares: importancia de la tipificación genética de las cepas en su evaluación e interpretación

OBJETIVO: El propósito del presente estudio ha sido describir 5 microepidemias de tuberculosis en centros escolares, establecer los factores de riesgo relacionados con su aparición, valorar la predicción de infección mediante la estrategia de círculos concéntricos y analizar la utilidad de la tipificación genética de cepa en su interpretación.

MATERIAL Y MÉTODOS: Se presenta el estudio de 5 brotes de tuberculosis, y su estudio convencional de contactos, ocurridos en 2 guarderías y 2 institutos de enseñanza media entre 1998 y 2005. Los contactos se estratificaron según el grado de convivencia siguiendo el sistema de círculos concéntricos. Se identificaron los perfiles genéticos (RFLP –polimorfismo de longitud de los fragmentos de restricción– IS6110) de las cepas disponibles y se cotejaron los resultados con el estudio de contactos para interpretar la transmisión de la infección.

RESULTADOS: Analizamos 5 brotes. En el primero estudiamos a 85 contactos; en el segundo, a 519; en el tercero, a 116; en el cuarto, a 655, y 102 en el quinto. La prevalencia de infección fue del 31, el 29, el 66, el 37,6 y el 32%, respectivamente. Se detectaron casos secundarios de enfermedad activa: 9 en el primero, 16 en el segundo, 5 en el tercero, 6 en el cuarto y 13 en el quinto. El análisis mediante RFLP identificó la coincidencia genética de todas las cepas en 3 brotes, y en el cuarto evidenció la existencia de al menos 2 cepas implicadas en su desarrollo. En los brotes 2, 3 y 5 encontramos una asociación importante entre el grado de convivencia y las probabilidades de infectarse ($p < 0,05$). En todos los brotes el riesgo relativo de infectarse y enfermar se asoció con la intensidad de la exposición.

CONCLUSIONES: El estudio de contactos basado en círculos de riesgo predice la probabilidad de infección. La RFLP permite aclarar vías de transmisión complejas que no son detectables mediante el estudio convencional de contactos.

Palabras clave: *Tuberculosis. Microepidemia. Brote. Escuela. RFLP.*

Introduction

Despite advances in recent years, tuberculosis continues to be a health problem in developed countries. The fundamental goal of tuberculosis control programs is the early diagnosis and effective treatment of affected

individuals. Systematic contact tracing is a secondary objective of these programs, since it allows detection of new cases of disease and recently infected individuals who can be treated prior to becoming new sources of infection.[1] These processes must be especially rigorous in the case of outbreaks or microepidemics.

In Galicia, Spain, the incidence of the disease is higher than in other Spanish autonomous communities (37.9 per 100 000 inhabitants in 2004), although the decline (7.9%) in this rate is greater than in other regions.[2,3] In this situation—an incidence that while in progressive decline still remains high—children and teenagers without immunity to the tuberculosis bacillus are especially susceptible, thereby facilitating the appearance of outbreaks or microepidemics when they are exposed to contagious individuals with tuberculosis.

The Galician Program for the Prevention and Control of Tuberculosis (Programa Galego de Prevención e Control da Tuberculose) located 1 of the 7 tuberculosis units in our hospital to serve close to 500 000 inhabitants.[3] Since 1996 all cases of tuberculosis in the area have been identified by active case finding and recorded in a central database with the capacity to alert to the development of microepidemics. Since that time, 5 outbreaks have been detected in schools and nurseries.

The aims of the present study were as follows: *a)* to analyze the characteristics of these microepidemics; *b)* to identify risk factors associated with their appearance; *c)* to assess the predictive capacity of contact tracing based on a concentric circle approach; and *d)* to interpret the mechanisms of transmission of the disease using the technique of restriction fragment length polymorphism (RFLP).

Material and Methods

We performed a prospective observational study of the microepidemics of tuberculosis detected in the period 1995-2005 in schools and nurseries in the area of A Coruña, Spain. We considered as a microepidemic, or epidemic outbreak, the appearance of 3 or more cases of tuberculosis related in space and time, or the diagnosis of at least 2 cases of disease generated by the same index case.[1] The study was performed under the conditions of the program for the control of tuberculosis in Galicia.[3]

Methods

Cases of tuberculosis were defined as those in which a positive culture was obtained with identification of *Mycobacterium tuberculosis* complex, or in which there were clinical, radiological, or anatomical findings indicative of tuberculous disease and a favorable response to treatment. The index case was considered as the first patient diagnosed and the source or true index case as that which most probably acted as the origin of the infectious outbreak. Contacts were classified as all individuals who had shared enclosed spaces with an individual with tuberculosis.

Following detection of index cases, contact tracing was carried out according to previously established procedures.[1,4] The conventional study was planned according to the concentric circles or stone-in-the-pond principle.[5] Contacts were stratified according to degree of contact with index cases in schools, families, or social settings. Circle 1 (C1), containing individuals at the highest risk, corresponded to subjects whose daily contact with the index case was greater than 6 hours; circle 2 (C2) contained those who had daily contact with the index case but for a period of less than 6 hours; and circle 3 (C3) contained sporadic contacts.[4]

The tuberculin test was performed with 2 tuberculin units of purified protein derivative RT-23 and induration was measured at 48 to 72 hours. Tuberculous infection was considered when the diameter of induration was at least 5 mm, independently of prior antituberculosis vaccination. A chest radiograph was performed in individuals with a positive tuberculin test and in cases with a negative result the test was repeated 3 months later. Following completion of the study, primary chemoprophylaxis, treatment of the latent infection, or antituberculosis treatment were recommended according to the protocol used in the program.[3]

In patients from whom mycobacterial cultures were obtained, the DNA fingerprint was identified by RFLP (RFLP-IS61190) using a previously described protocol.[6] The results were compared with those of conventional contact tracing to interpret the mechanism of transmission in each specific outbreak.

Statistical Analysis

We analyzed the overall relative risks of infection and disease for the contacts according to the risk circle to which they belonged, discarding from the analysis those who did not complete the study.[1,22] We also carried out a stratified analysis of the group of schoolchildren, excluding the adult population to avoid bias created by greater heterogeneity and higher baseline prevalence of infection. We used the χ^2 test and Fisher test to compare the number of cases between risk circles in each contact study using the Epidat program version 3.1. We determined the relative risk (RR) and its respective 95% confidence interval (CI). Values of *P* less than .05 were considered statistically significant.

Results

In the period studied, we detected 5 microepidemics in 2 day nurseries and 2 high schools. Table 1 shows the characteristics of the source cases.

TABLE 1
Characteristics of the Source Cases for Each Outbreak

Outbreak	Center	Age, y	Role in the Center	Respiratory Symptoms, mo	Smear	Culture	Cavitation in Chest Radiograph
1	Day nursery	24	Support staff	8	+	+	+
2	High school	17	Student	12	+	+	+
3	High school	17	Student	1.5	+	+	+
4	High school	17*	Student*				
5	Day nursery	24	Childcare	4	+	+	+

*Probably 2 source cases, 1 the same as in outbreak 3.

Outbreak 1

Outbreak 1 was detected in 1998 in a day nursery for children aged 1 to 4 years. The index cases were 2 girls diagnosed on the same day (which raised the alarm regarding a possible outbreak). The source case was a support worker diagnosed following the outbreak (Table 1) who had stopped working at the nursery 3 months earlier. We identified 92 contacts and 85 completed the study; 69.4% were students (Table 2). C1 contained family members in the same home and classmates (children aged 2 to 3 years); C2 included children from another class (less than 2 years old) and teachers from the school; and C3 contained students of nursery care doing training placements in the nursery.

Of the 85 contacts studied, we diagnosed 26 (30.6%) as being infected. In C1, 33.3% of the children were infected and 8 had developed the disease. In C2, 26.3% of cases were infected; most were teachers and the only child who was infected developed the disease. In C3, 30% of the contacts were infected but none developed the disease.

Half of the contacts with a positive tuberculin test were children attending the nursery. The cases that developed the disease were exclusively children attending the nursery; 8 were members of the class in which the source case worked and 1 was from another class, representing a prevalence of tuberculosis within the group of nursery children of 15.3%. All cases had radiological evidence of tuberculosis: 3 cases had consolidation alone, 3 had only lymph node involvement, and the remaining 3 had consolidation with ipsilateral hilar lymph node involvement. Mycobacterial cultures were not obtained in the processed samples.

The overall analysis did not reveal significant differences in the probability of infection according to the circles of risk. However, considering only the children, the risk of infection in C1 was 7.66 times greater (95% CI, 1.07-55.07; $P<.001$), with an RR of 7.33 for development of the disease (95% CI, 10.97-55.53; $P<.001$) compared with those in C2.

Outbreak 2

Outbreak 2 was detected in 1999 in a high school for children aged 13 to 17 years. The index and source case was a student in the school who was a member of a social group that undertook extracurricular activities. He was smear positive and had a long history of respiratory symptoms. We identified 650 contacts, of whom we were able to evaluate 519. They were classified as follows: C1 included individuals from the same home, children from the same class, and habitual friends of the index case; C2, children in the same year group but from different classes and individuals who undertook extracurricular activities with the index case (extra tutoring and dancing); and C3, other children in the school and staff (Table 3). The overall rate of infection in the outbreak was 29.3%. Sixteen cases of tuberculous disease were diagnosed: 8 in C1, 4 in C2, and 4 in C3 (all were schoolchildren). Eleven patients had pulmonary tuberculosis, 1 with cavitation; in 2 cases there was pleural involvement and in 3 the diagnosis was primary tuberculosis with mediastinal lymph node involvement.

TABLE 2
Contacts in Outbreak 1

Circle	Group	No.	Infected*	Active Disease
C1	Schoolchildren	36	12 (33.3%)	8 (22.2%)
	Family members	1	1 (100%)	0 (0%)
	Total C1	37	13 (35.1%)	8 (21.6%)
C2	Schoolchildren	23	1 (4.3%)	1 (4.3%)
	Teachers	15	9 (60%)	0 (0%)
	Total C2	38	10 (26.3%)	1 (2.6%)
C3	Childcare students	10	3 (30%)	0 (0%)
	Total C3	10	3 (30%)	0 (0%)
Contacts studied		85	26 (30.6%)	9 (10.6%)

*Includes individuals with the disease.

TABLE 3
Contacts in Outbreak 2

Circle	Group	No.	Infected*	Active Disease
C1	Schoolchildren	30	26 (86.7%)	5 (16.7%)
	Family members	5	5 (100%)	1 (20%)
	Friends	3	3 (100%)	2 (66.7%)
	Total C1	38	34 (89.5%)	8 (21.1%)
C2	Schoolchildren	41	23 (56.1%)	2 (4.9%)
	Social activities	35	18 (51.4%)	2 (5.7%)
	Total C2	76	41 (53.9%)	4 (5.3%)
C3	Schoolchildren	380	64 (16.8%)	4 (1%)
	Teachers	25	13 (52%)	0 (0%)
	Total C3	405	77 (19%)	4 (1%)
Contacts studied		519	152 (29.3%)	16 (3%)

*Includes individuals with the disease.

Only 1 was smear positive, although a positive culture was obtained in 6 cases.

The schoolchildren in C1 had an RR of infection of 1.54 (95% CI, 1.14-2.01) compared with C2 ($P<.01$) and 5.15 (95% CI, 3.6-7.5) compared with schoolchildren in C3 ($P<.01$). The RR for development of the disease in schoolchildren classified as C1 was 3.41 (95% CI, 0.71-16.43) compared with C2 ($P=.09$) and 15 (95% CI, 4.5-55.9) compared with those in C3 ($P<.01$). Genetic analysis of the strains corresponding to the 6 patients in whom a positive culture was obtained revealed that they shared the same genetic fingerprint.

Outbreak 3

Outbreak 3 was declared in February 2004 in a high school. The index and source case was a 17-year-old male student who was diagnosed 6 weeks after onset of symptoms (Table 1). An initial study (Table 4) included 116 contacts (of whom 73.3% were students at the school). Individuals living in the home of the index case, children from the same class, members of the soccer team to which the index case belonged, and habitual friends were classified as C1; teachers and children who shared the same classroom at some time during the week were classified as C2; and children from the same year group as the index case but in other classes were classified as C3 (Table 4). The overall rate of infection for the highest risk group (C1) was 94.1%.

Five cases of tuberculosis were diagnosed (4 in C1 and 1 in C2); none were smear positive and 3 had positive sputum cultures. RFLP revealed that all isolates were genetically similar.

Outbreak 4

Outbreak 4 occurred in the same school as outbreak 3, and was identified 3 months after completion of the study for outbreak 3. The alarm was raised upon detection of 6 new cases of the disease, of which 3 had indicative symptoms at the time of diagnosis and had cavitation in the chest radiograph; only 1 was smear positive, 3 had positive cultures, and there was 1 case of pleural tuberculosis. In 1 of those 6 cases, a boy in the same class as the index case, a positive result had been obtained in the tuberculin test carried out following declaration of outbreak 3 but chemoprophylaxis had not been used as indicated; the remaining 5 cases did not have an obvious relationship with the risk groups established for the previous outbreak. A new study was therefore initiated to include all members of the school and coincided with a new school year. In this second study we identified 699 contacts, of which 655 completed the study (Table 5). Among those who were lost to follow-up, 39 were in the low-risk group. C1 included 133 classmates of any of the new cases detected; C2 included schoolchildren in classes that did not contain any of the new cases and 56 teachers; and C3 included the 83 schoolchildren who had just joined the school as they all formed part of the new school intake. Since this last group had not had prior contact with the school, the rate of infection reflected the prevalence of infection in the population served by the school, and C3 could be interpreted as a control group (Table 5). In those subjects in whom a negative result had been obtained in the tuberculin test performed in the previous study the complete programmed study was repeated, and in those in whom evidence of infection was obtained, all relevant tests to rule out disease were performed.

The rate of infection was significantly higher in the group of children who were classmates of individuals with the disease (57.9%) than in those who did not share a classroom with a case of tuberculosis (33.9%) or in children who had just joined the school (3.6%). The schoolchildren in C1 had an RR of infection of 1.70 (95% CI, 1.39-2.08) compared with C2 (*P*<.01) and 16 (95% CI, 5.2-49.1) compared with C3 (*P*<.01). No new cases of tuberculosis were detected in addition to the 6 cases detected initially.

RFLP analysis of the 3 cultures obtained revealed genetic similarity in 2 isolates (genetically identical to those of outbreak 3), while the remaining isolate was genetically different. These findings indicate that at least 2 strains were involved in the outbreak.

Outbreak 5

Outbreak 5 was declared in 2005 in a day nursery. The index case and source of the infection (Table 1) was a 24-year-old childcare worker with a history of primary tuberculosis at the age of 1 year that had been correctly treated, and who had lived with an individual with

TABLE 4
Contacts in Outbreak 3

Circle	Group	No.	Infected*	Active Disease
C1	Schoolchildren	30	28 (93.3%)	2 (6.6%)
	Family members	3	3 (100%)	0 (0%)
	Soccer team	14	13 (92.8%)	1 (7.1%)
	Friends	4	4 (100%)	1 (25%)
	Total C1	51	48 (94.1%)	4 (7.8%)
C2	Schoolchildren	28	16 (47.1%)	1 (3.6%)
	Teachers	10	8 (80%)	0 (0%)
	Total C2	38	24 (63.2%)	1 (2.6%)
C3	Schoolchildren	27	5 (18.5%)	0 (0%)
	Total C3	27	5 (18.5%)	0 (0%)
Contacts studied		116	77 (66.4%)	5 (4.3%)

*Includes individuals with the disease.

TABLE 5
Contacts in Outbreak 4

Circle	Group	No.	Infected*	Active Disease
C1	Schoolchildren†	133	77 (57.9%)	6 (4.5%)
	Total C1	133	77 (57.9%)	6 (4.5%)
C2	Teachers	56	36 (64.3%)	0 (0%)
	Schoolchildren‡	383	130 (33.9%)	0 (0%)
	Total C2	439	166 (37.8%)	0 (0%)
C3	Schoolchildren§	83	3 (3.6%)	0 (0%)
	Total C3	83	3 (3.6%)	0 (0%)
Contacts studied		655	246 (37.6%)	6 (0.9%)

*Includes individuals with the disease.
†Classes containing individuals with the disease.
‡Classes without individuals with the disease.
§Control class.

TABLE 6
Contacts in Outbreak 5

Circle	Group	No.	Infected*	Active Disease
C1	Schoolchildren	30	10 (33.3%)	9 (30%)
	Family members	3	3 (100%)	1 (33.3%)
	Total C1	33	13 (39.4%)	10 (30.3%)
C2	Schoolchildren	35	6 (17.1%)	2 (5.7%)
	Teachers	11	5 (45.5%)	0 (0%)
	Total C2	46	11 (24.4%)	2 (4.3%)
C3	Schoolchildren	15	2 (13.3%)	1 (6.7%)
	Total C3	15	2 (13.3%)	1 (6.7%)
Contacts studied		94	26 (27.6%)	13 (13.8%)

*Includes individuals with the disease.

tuberculosis 3 years earlier. We identified 94 contacts, all of whom completed the study (Table 6). C1 included individuals living with the index case and children aged up to 1 year who were cared for by the index case; C2 contained children of other ages who had contact with the index case in the dining hall; and C3 included those children who had only had contact with the index case 1 day per week. The overall rate of infection was 32%. Thirteen cases of the disease were diagnosed: 12 children from the nursery and another individual, the partner of the index case, who had been treated for tuberculosis 5 years earlier. Among the children who had both pulmonary and lymph node involvement, tuberculosis bacilli were isolated by culture of gastric juice in 3 cases. The cultured strain was

resistant to streptomycin, as was also observed for the index case.

There were significant differences in the probability of infection according to the circles of risk. The risk of infection in the children in C1 was much higher than in those who were less exposed, with an RR of 1.94 (95% CI, 0.8-4.72) compared with C2 (P=.013) and 2.5 (95% CI, 0.62-9.99) compared with C3 (P=.15). Likewise, the probability of developing the disease was very high in children in C1, with an RR of 5.25 (95% CI, 1.25-22.44) compared with C2 (P<.01) and 4.5 (95% CI, 0.63-37.52) compared with C3 (P=.07). In this outbreak, RFLP confirmed the involvement of a single strain that was genetically identical to the strain that had caused the disease in the individual who lived with the index case 3 years earlier, who at the time of the outbreak had recurrence with the same strain.

Discussion

Outbreaks or microepidemics of tuberculosis represent a special risk for public health. They have been described in very different settings, such as hospitals,[7] bars,[8] churches,[9] and even travelers on a bus.[10] Schools are special environments in which shared living space and close proximity facilitate the appearance of epidemic outbreaks of tuberculosis.[11] However, this situation has not been adequately recognized in Spain, as shown by a recent study of Catalonia, where, with a mean incidence of 0.40 outbreaks per 100 000 inhabitants per year, only 1 school-based outbreak was detected from among the 27 outbreaks studied.[12]

Tuberculosis in infant populations provides an up to date reflection of active transmission of the disease in the community. Thus, children represent an excellent indicator for control programs, especially in countries with a low incidence of the disease. Furthermore, the close proximity of school groups favors the development of tuberculosis outbreaks. Contributing factors include sustained contact, inadequate classroom ventilation, and delayed diagnosis.[13,14] In the case of microepidemics, the diagnostic delay is usually particularly long.[15] Our findings confirm this observation, since in all except 1 outbreak there was a very long diagnostic delay (close to a year in 1 case), thereby favoring exposure and microepidemics.[11,16,17] The delay is usually due to a failure to recognize symptoms and to diagnostic errors, interpreting tuberculosis as a community-acquired pneumonia, as occurred in the index cases from outbreaks 1 and 2. These findings indicate that tuberculosis is a disease in which a low level of clinical suspicion hinders diagnosis.[18] Increasing the level of suspicion among professionals and the general population is a priority for tuberculosis control programs in areas where the disease is in decline.

Children present forms of tuberculosis that are not usually very contagious. Preschool-age children usually show no development of pulmonary cavitation, have negative sputum smears, and do not have sufficient cough strength to aerosolize tuberculosis bacilli, elements which also hinder diagnosis.[14,19] This is supported by the cases studied in outbreak 5, since we only obtained microbiological diagnosis in 3 out of 21 children (14.3%),

in all cases through culture of gastric juice, a technique for which the diagnostic yield in children is not well accepted.[20] In contrast, older children and adolescents tend to be more contagious, and can occasionally be superspreaders.[19,21] In our study, we were able to confirm the importance of sputum smears and culture at these ages.

The contact tracing strategy employing concentric circles of risk[4] is not widely used in school settings. However, the results of this study demonstrate that it is an epidemiological approach that provides an acceptable prediction of risk, as demonstrated in outbreaks 1, 2, and 5. In outbreak 3, the conventional study did not predict the presence of new cases of disease outside the circles initially studied, a finding which is partly explained by the presence of at least 2 strains in the school environment. We cannot rule out the possibility that the source of the disease could be found elsewhere, separate from the school. Nevertheless, once the new outbreak was declared, we found that involvement with the school was a risk factor for infection or development of the disease, since the prevalence of infection in the students who had recently joined the school was low and similar to that expected for the population in that age group.

Analysis of the outbreaks in nurseries confirmed the speed and ease with which the infection progresses to disease in preschool children, with a very high risk of developing the disease in infected children irrespective of the level of contact. We therefore believe that in preschool-age children contact tracing should be extended to all children within the nursery from the outset. In these cases, teachers and childcare staff are usually the source of the disease,[11,16,22] unlike in outbreaks occurring in schools for older children, where this is less frequent.[17,22] As a result, it has been proposed that obligatory tuberculosis screening should be performed in individuals who have close contact with infants and young children.[20] Another risk factor is the length of exposure and contact with the contagious case,[22] as shown in our study, where individuals in C1 had a higher probability of infection.

A factor that can complicate treatment and chemoprophylaxis is infection with bacilli resistant to an antituberculosis drug. We could verify this situation (previously reported for isoniazid in a school-based epidemic[24]) in 1 of the outbreaks, where we isolated a strain that was resistant to streptomycin. Although it has limited therapeutic implications, this represents a finding that has not been described previously in a school-based microepidemic.

RFLP analysis provided very valuable data for the epidemiological characterization of the microepidemics. Use of this technique allowed us to confirm that a single strain was responsible in the patients with positive sputum culture in 2 of the outbreaks. The exception was outbreak 4, where at least 2 different strains were involved, 1 identical to the strain in outbreak 3 and another different strain. This confirms that RFLP is an especially useful tool for determining complex routes of transmission that are not detectable with conventional contact studies.[25] The data obtained in outbreak 5 served to identify exogenous reinfection in cases of recurrence.[26,27] In this case, reinfection of an individual who previously had the disease led to an outbreak in a particularly susceptible group. This

finding once again highlights the appropriateness of more than 1 course of chemoprophylaxis throughout the individual´s lifetime if there is more than 1 risk exposure.

This study had certain limitations. In some cases, bacteriological confirmation of the disease was not obtained (particularly in children and extrapulmonary forms), and as a result, the number of active cases of tuberculosis could be overestimated. In addition, with the exception of outbreak 5, not all contacts completed the study and we therefore do not know if they were infected. Although possible, it is less likely that they would have developed the disease, given the characteristics of the case finding used in our control program. Furthermore, it is likely that if another definition of outbreak were used, such as that of the Spanish national epidemiological surveillance network,[28] which defines it as the appearance of 1 or more cases of tuberculosis derived from the same index case in a period of 1 year, the number of outbreaks detected in the study period may have been higher.

In conclusion, this study shows that in the analysis of microepidemics of tuberculosis, contact tracing based on circles of risk accurately predicts the risk of infection and that the strains isolated from patients with the disease should be analyzed by RFLP, since in that way a clearer picture of the infection can be obtained and it is even possible to identify routes of transmission that cannot be identified by contact tracing.

Acknowledgments

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