

## Descriptive Study of Human and Bovine Tuberculosis in Querétaro, México

FELICIANO MILIÁN,<sup>1,2\*</sup> LUISA M. SÁNCHEZ,<sup>2</sup> PAOLA TOLEDO,<sup>2</sup> CAROLINA RAMÍREZ,<sup>1</sup> AND MARCO A. SANTILLÁN<sup>1</sup>

Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, CENID-Fisiología, Km. 1 Carretera a Colón, Ajuchitlán, Qro.<sup>1</sup> Facultad de Ciencias Naturales, UAQ, 16 de Septiembre No. 63 Ote. Centro, Querétaro, Qro., México, C. P. 76000.<sup>2</sup>

\*Corresponding author: E-mail milianl@sunserver.uaq.mx

**ABSTRACT.** A 5-year retrospective study (1992-1996) to look at the situation of human tuberculosis was conducted in Querétaro, México. Also, a 6-month study to determine the frequency of gross lesions in dairy cattle at slaughter, and a short experiment to evaluate the effect of sodium borate in the survival of *M. bovis* in lesions were carried out. The number of cases were 114 in 1992, 211 in 1995, and 174 in 1996. Possible risk factors were: overcrowding, under-nutrition, previous cases of TB in the family, concurrent *Diabetes mellitus*, poor personal hygiene, smoking, and alcohol abuse. Eighty percent of the cases were pulmonary. The number of cases increase with age, from 5% in patients 10-year old or younger to 42% in patients 50-year old or older. Seventy-two percent were cured, and only 6% die. Persistent coughing was by far the most observed clinical symptom. From 112 acid-fast negative samples, 8.9% were positive by culture. From 1,201 carcasses revised at slaughter, 17% presented TB-gross lesions. Ninety-six percent were localized lesions involving frequently a single organ, mostly retropharyngeal, mediastinal, mesenteric and mandibular lymph. From 102 lesions, 95% were TB-compatible, and 79% were positive to isolation of *M. bovis*. Most affected animals were female >2 years old. It was observed that keeping lesions in a 6% sodium borate solution does not affect the diagnosis of *M. bovis* by culture after 150 days.

**Key words:** Tuberculosis, *Mycobacterium bovis*

**RESUMEN.** Un estudio retrospectivo de 5 años (1992-1996) se realizó para analizar la situación de la tuberculosis (TB) en el Estado de Querétaro, México. Al mismo tiempo se hizo un estudio de 6 meses para determinar la frecuencia de lesiones macroscópicas en ganado lechero en el rastro, y un experimento corto para evaluar el efecto del borato de sodio en la sobrevivencia de *M. bovis* en tejidos. El número de casos fue de 114 en 1992, 211 en 1995 y 174 en 1996. Los posibles factores de riesgo fueron: sobrepoblación de vivienda, desnutrición, historia de casos previos de TB en la familia, concurrencia de *Diabetes mellitus*, pobre higiene personal, el hábito de fumar y el abuso de alcohol. Los casos de TB pulmonar alcanzaron el 80%. El número de casos se incrementó del 5% en pacientes de 10 años de edad o menores a 42% en pacientes de 50 años o mayores. La tos persistente fue el síntoma clínico más observado. De 112 muestras negativas a la prueba de ácido-alcohol resistente, 8.9% fueron positivas al cultivo. De 1,201 canales de bovino revisadas, 17% presentó lesiones macroscópicas sospechosas de TB, principalmente de tipo localizado en nódulos linfoides retrofaríngeos, mediastínicos, mesentéricos y mandibulares. El análisis histopatológico mostró que el 95% de las muestras compatibles con TB, y del 79% se aisló a *M. bovis*. Los animales afectados fueron en su mayoría mayores de 2 años de edad. Se observó que el guardar tejido con lesión en borato de sodio al 6% no afecta el diagnóstico de *M. bovis* por cultivo por periodos de al menos 150 días.

**Palabras clave:** Tuberculosis, *Mycobacterium bovis*

### INTRODUCCIÓN

Tuberculosis (TB) is one of the main causes of death all over the world. In 1995, TB killed 3 million people, of whom 6% were less than 15 years old.<sup>7,15</sup> In developed countries, the incidence of TB decreased until recently; however, from 1985 through 1993 in the United States the morbidity of TB increased 14%. The most important factors reported for this increase are: infections with the

HIV virus, occurrence of TB in foreign-born persons, the emergence of drug-resistance strains, and the transmission of *M. tuberculosis* in congregate settings such as health-care facilities, correctional facilities, drug-treatment centers, and homeless shelters.<sup>10</sup>

Bovine TB caused by *Mycobacterium bovis* is an important veterinary disease that also affects humans. As it has been reported,<sup>1</sup> there is a four-way relationship between tuberculosis in human and cattle: human TB caused



by *M. tuberculosis*, human TB caused by *M. bovis*, TB in cattle caused by *M. bovis*, and TB in cattle caused by *M. tuberculosis*. In the last decade, AIDS has increased the number of individuals susceptible to infection with *M. bovis*, which currently is a serious concern.<sup>5</sup> Pasteurization has decreased the incidence of human TB by *M. bovis* in developed countries; however, TB by this species remains an important veterinary disease as well as public-health problem in some parts of the world.<sup>5,6</sup> *M. bovis* is blamed for about 7,000 cases of human TB each year in Latin America.<sup>11,15</sup>

The present study was conducted in order to look at the situation of both human and bovine tuberculosis in the State of Querétaro, including an analysis of the survival of *M. bovis* in a solution commonly used to transport lesions to the laboratory, and an evaluation of the efficacy of the acid-fast diagnostic technique used in the State.

## MATERIALS AND METHODS

To evaluate the situation of human and bovine tuberculosis in the state of Querétaro, México, two main studies and a short experiment were conducted. The first study was a 5-year retrospective study (1992-1996) of human cases of TB in the state. The second study consisted of a 6-month prospective study for detection of gross lesions in dairy cattle at slaughter. The experiment consisted in evaluating the effect of sodium borate in the survival of *M. bovis* in tissue samples.

**Human TB.** Information about human cases of TB was obtained for a 5-year period, 1992 to 1996. Information was for all cases observed in the state, which has a population of 1.5 million. TB-cases were defined as patients with sputum positive to the presence of acid-fast *Mycobacterium*. Information obtained from case-records included individual characteristics such as origin, age, sex, personal hygiene and nutritional status. We looked at social variables such as housing, occupation (partially available), and number of individuals in the house, host habits, smoking and alcohol abuse, clinical variables such as case type (new or recurrent), previous cases of TB in the family, and concurrence of *Diabetes mellitus* and AIDS. Information about clinical symptoms (cough, fever, weight loss, and pain in thorax), presentation of the disease according to the organ affected (lung, meninges, bone, lymph node, kidney, and other), and final outcome of the case (cure, quit treatment, death) was also obtained.

Risk factors were defined as follows: overcrowding, when 3 or more persons lived or sleep in a single-room house; under-nutrition, when the diet was considered poor in proteins and carbohydrates; poor hygiene when persons manifest not following the basic rules of personal hygiene (more than two showers a week, washing their hands before meals and after going to the toilet, changing clothes regularly); poor housing, when house building material

was cartoon, leaves of trees, grass, and the floor was pure soil; Previous TB in the family, when the cases reported other cases in the family in the past; Smoking, when the case reported smoking more than one package of cigarettes a week, and alcohol abuse, when the case reported drinking regularly, every weekend.

Crude incidence risks per year were obtained using the 1995 state population as the average population at risk. Analysis of variables is presented in frequency tables. To evaluate distribution of cases by age, this variable was categorized in six 10-year groups, the last group included patients 50 years old and older. In order to evaluate the accuracy of the routine diagnostic procedure used by laboratories in the state (presence of acid-fast mycobacteria), all 181 sputum samples collected between November, 1996 and June, 1997 (69 positive and 112 negative) were cultured in selective medium (Lowenstein-Jensen and Stonebrink) to facilitate the growth of both, *M. tuberculosis* and *M. bovis*.

**Bovine TB.** TB-suspicious lesions were obtained from dairy cattle by careful inspection of carcasses in slaughterhouses for a period of 6 months. Sampling was by convenience; all dairy animals slaughtered the day of the visit during the sampling period were included in the study. Carcasses were examined thoroughly but focus was on lymph nodes: mandibular, retropharyngeal, and parotid in the head; tracheobronchial and mediastinal in the thorax; and hepatic and mesenteric in abdomen. All lesions observed were carefully removed from the carcass and placed in plastic bags in a cooler with ice, and then frozen until processed in the laboratory. Infection of lesions was confirmed by isolation of *M. bovis* by culture in a selective medium (Stonebrink). Culture was performed following a standard procedure.<sup>12</sup> Species identification of the *Mycobacterium* was accomplished by growth characteristics, colony morphology, and conventional biochemical tests. Epidemiological information about affected animals such as age, sex, body condition, organs affected, and type of TB (localized/generalized) was obtained during carcass inspection, that about origin of the affected animals was obtained by informal interview to cattle dealers. Data were processed by a descriptive analysis and the results presented in descriptive tables.

**Survival of *M. bovis*.** TB-suspicious lesions are usually placed in a 6% sodium borate solution for shipping and storage until processing the samples in the laboratory. For many reasons tissue lesions remain in the solution for weeks or even months before processing. There are reports in the literature, however, that sodium borate reduces the viability of *M. bovis*.<sup>3,4</sup> To evaluate this possibility, each lesion from 10 lymph nodes was divided into 10 sections and, except for section one, which remained fresh, all sections were placed in glass containers with a 6% sodium borate solution. Then, culture was attempted in a selective medium (Stonebrink) at different time periods for two replicates of each section as follows: day 0 fresh sample, and

then treated samples at days 2, 4, 7, 15, 30, 60, 90, 120 and 150 post-treatment. Identification of *M. bovis* was performed by the conventional biochemical tests.

## RESULTS

**Human TB.** The total number of cases of human TB in the 5-year period for the state of Querétaro was 799. The annual incidence from 1992 to 1996 were 114, 120, 180, 211, and 174, respectively.

Information was not complete for all variables included in the study, therefore, totals in tables differ according to the factor involved. Pulmonary was by far the most common presentation of the disease, with 80% of a total of 265 cases (Table 1). In order of frequency, the possible risk factors were: overcrowding, under-nutrition, previous cases of TB in the family, concurrent *Diabetes mellitus* and poor hygiene.

The proportion of cases increased with age (n=759), from 5% in patients 10-years old or younger to 40% in patients 50-years old or older (Fig. 1). Among cases for which information about occupation was available (n=148), housewife (38%), farmer (13%), and student (11%) were the occupations most frequently reported (Table 2). From 293 cases with ending-outcome information, 72% were cured, and only 6% ended on death (Table 3). Finally, from 674 cases, it was found that 85% were new cases, and that persistent coughing was by far the most frequent symptom observed, 51% (Table 4).

From the 181 human-sputum samples processed by culture to evaluate the accuracy of the acid-fast diagnostic approach followed by laboratories, 10 (8.9%) were culture-positive, from the acid-fast negative group (n=112). Surprisingly, from the acid-fast positive group (n=69), only 11 (15.9%) were positive by culture. At least 3 of these isolates were identified as *M. bovis* by biochemical tests.

**Bovine TB.** Findings of the study conducted to deter-

Table 1. Distribution of cases by type of TB and possible risk factors\*. A five years retrospective study, 1992-1996, Querétaro, México.

Factor	Type of tuberculosis											
	Pulmonary	%	Meningeal	%	Nodular	%	Miliar	%	Other	%	Total	%
Overcrowding	77	36	4	31	5	31	2	17	8	67	<b>96</b>	36
Under-nutrition	23	11	5	38	2	12	6	50	0	0	<b>36</b>	14
Previous TB in the family	23	11	2	15	3	19	0	0	1	8	<b>29</b>	11
<i>D. mellitus</i>	26	12	1	8	0	0	1	8	0	8	<b>28</b>	11
Poor hygiene	24	11	0	0	1	6	0	0	0	0	<b>25</b>	9
Smoking	13	6	0	0	3	19	2	17	1		<b>19</b>	7
Alcohol	12	6	0	0	0	0	1	8	2	0	<b>15</b>	6
Poor housing	9	4	0	0	0	0	0	0	0	0	<b>9</b>	3
AIDS	5	2	1	8	2	12	0	0	0	17	<b>8</b>	3
<b>Total</b>	<b>212</b>		<b>13</b>		<b>16</b>		<b>12</b>		<b>12</b>		<b>265</b>	
<b>%</b>	<b>80</b>		<b>5</b>		<b>6</b>		<b>4</b>		<b>5</b>			

\* Information about risk factors was available for 265 cases.

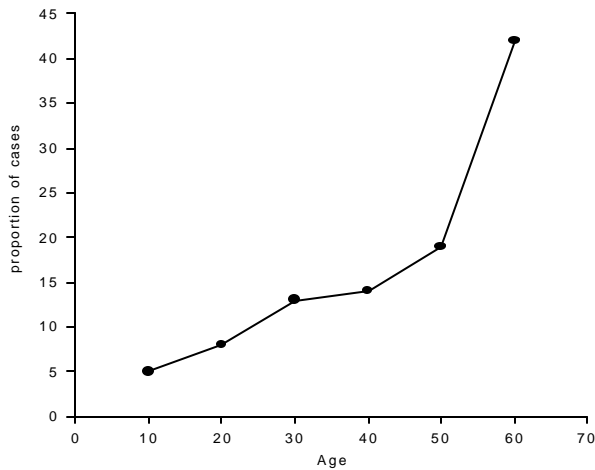


Figure 1. Proportion of cases of human TB per age-group. Age in years was categorized in 10-year groups with the last group including patients 50-years old and older. A five years retrospective study, 1992-1996, Querétaro, México.

mine the frequency of dairy cattle with TB-suspicious lesions at slaughter are summarized in Tables 5 and 6. From a total of 1,201 carcasses of dairy animals inspected in 60 days in a period of 6 months, 209 (17%) presented gross lesions suspicious of TB. The average number of lesions per day was 3.5, with a range from 2.6 to 4.2. As shown in Table 6, lymph nodes were by far the most affected organs with 171 cases (85%). From these, the retropharyngeal (35%), the mediastinal (27%), the mesenteric (10%), and the mandibular (9%) were the specific lymph nodes most frequently affected.

From the 209 TB-suspicious lesions, a random sample of 102 were selected and processed by culture for isolation of *Mycobacterium*, and for diagnosis by histopathology; 81 (79 %) were positive to isolation, and 97 (95 %) were positive to lesions compatible with TB by histopathology. Affected animals were mostly female (83%), more than 2 years of age, and in fair to good body condition scores (66%). Ninety-six percent of the cases presented local lesions affecting a single organ.

**Survival of *M. bovis*.** Diagnosis of *M. bovis* by culture is not affected if lesions remain in sodium borate for up to 150 days. Even though the number of colonies decreased (from 500 in the first 30 days to 10 colonies at 150 days) the diagnosis would still be positive because this is based on the presence and not on the number of colonies of *My-*

**Table 2.** Distribution of cases of human TB by occupation\* and possible risk factors. A five years retrospective study, 1992-1996, Querétaro, México.

Occupation	Possible risk factors								Total	%
	Over-crowding	Poor hygiene	Previous TB in the family	Under-nutrition	Concurrent <i>D. mellitus</i>	Smoking	Alcohol abuse	Poor housing		
Housewife	25	7	9	7	3	1	1	3	<b>56</b>	38
Farmer	7	9	0	1	0	0	1	1	<b>19</b>	13
Student	5	1	7	3	0	1	0	0	<b>17</b>	11
Construction worker	4	1	0	0	2	2	0	0	<b>9</b>	6
Teacher	0	0	2	0	1	3	1	0	<b>7</b>	5
Laborer	0	0	0	1	1	3	1	0	<b>6</b>	4
Employee	0	0	0	1	1	0	1	0	<b>3</b>	2
Other	10	3	2	1	6	3	5	1	<b>31</b>	21
<b>Total</b>	<b>51</b>	<b>21</b>	<b>20</b>	<b>14</b>	<b>14</b>	<b>13</b>	<b>10</b>	<b>5</b>	<b>148</b>	
<b>%</b>	34	14	13	9	9	9	7	3		

\*148 cases had information about occupation.

Table 3. Distribution of cases of human TB by final outcome\*. A five years retrospective study, 1992-1996, Querétaro, México.

Ending outcome									
Cure		Death		Abandoned treatment		Move to another clinic		Total	
Freq.	%	Freq.	%	Freq.	%	Freq.	%		
197	92	15	88	20	80	84	32		
16	7	2	12	5	20	16	6		
<b>213</b>		<b>17</b>		<b>25</b>		<b>38</b>		<b>293</b>	
72%		6%		8%		13%			

\*293 cases had information about final outcome.

Table 4. Distribution of cases of human TB by type of case and the most frequently observed clinical symptom\*. A five years retrospective study, 1992-1996, Querétaro, México.

Type of case	Symptoms										Total	%		
	Coughing		Fever		Hemophthisis		Weight loss		Pain in thorax				All five	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%			Freq.	%
New	310	54	88	15	52	9	39	7	21	4	63	11	<b>662</b>	85
Recurrent	32	32	10	10	22	2	2	2	4	4	31	31	<b>101</b>	15
<b>Total</b>	<b>342</b>		<b>98</b>		<b>74</b>		<b>41</b>		<b>25</b>		<b>94</b>		<b>674</b>	
%	51		14		6		12		4		14			

\* 674 cases had information about clinical symptoms.

Table 5. General statistics of a convenience sampling of TB-suspicious lesions of dairy cattle at slaughter. July-December, 1996, Querétaro, México

Month	Sampling days per month	Animals slaughtered	Animals with lesions	TB-suspicious lesions per month	TB-suspicious lesions per day
July	10	221	40	18	4.0
August	17	355	66	19	3.8
September	10	180	29	16	2.9
October	7	127	18	14	2.6
November	8	157	34	22	4.2
December	8	161	22	14	2.7
<b>Total</b>	<b>60</b>	<b>1,201</b>	<b>209</b>	<b>17</b>	<b>3.5</b>

**Table 6.** Frequency of organs affected with TB-suspicious lesions during carcass inspection of dairy cattle slaughtered in an abattoir in Querétaro, México.

Organs affected	Frequency and Proportion of organs affected		Specific lymph nodes affected, alone or in combination	
	Frequency	%	Frequency	%
Lymph node alone	171	85		
Retropharyngeal			131	35
Mediastinal			101	27
Mesenteric			36	10
Mandibular			34	9
Other			68	18
Other	30	15		
Generalized TB	8			
<b>Total</b>	<b>209</b>		<b>370</b>	

*cobacterium*. Nevertheless, the recommendation that samples be processed as soon as possible after collection to obtain the largest number of colonies possible, remains.

## DISCUSSION

Tuberculosis in humans presented an increasing pattern in the state of Querétaro for the period of 1992 to 1996. The increasing pattern of cases seems to be a reflection of the situation of the disease in the Country, where from 1996 to 1997 the number of cases increased, in some cases 100%, in 28 out of the 32 states.<sup>8</sup> It is possible, however, that this trend is the result of the publicity given to TB in the last 10 years; willingness to diagnose the disease may have increased in response.

The decrease in the number of cases in 1996 may be apparent, since no especial programs to reduce the disease had been implemented in that or in previous years. We believe this was because not all cases for that year had been collected at the time the study ended, even when we were told otherwise.

In relation to risk factors, overcrowding, undernutrition, poor housing, and poor personal hygiene, four of the risk factors most frequently detected in case-records are indicators of poor social and economical status. This confirms that TB is a "low-class" disease. Improving living conditions has to be an important component in any program to reduce TB in the human population. *Diabetes mellitus* is a disease that compromises the immune system, therefore, it is not surprising that a large proportion of individuals have both *Diabetes* and TB. If *Mycobacterium* persist longer in the environment, as a consequence of more

infected people or animals, the number of cases are expected to increase in response to a more susceptible population because of immune-compromising diseases, hereby the importance of eliminating the disease from the environment.

Susceptibility to TB has been reported to increase with age.<sup>13</sup> Our findings totally agree with those reports, the number of cases of TB was larger in the older group. However, it has also been reported that clinical disease may follow soon after initial infection with *Mycobacterium tuberculosis* or it might occur many years after primary infection, either through endogenous reactivation or after exogenous re-infection.<sup>14</sup> Consequently, an increasing number of cases in older patients do not necessarily mean more new infections at that age. As a matter of fact, it has been mentioned that the risk of developing TB is higher for adolescents and adults than among children,<sup>9</sup> but not age at infection has been clearly determined.

From the 112 acid-fast negative sputum samples culture to double-check the results from the laboratories, 10 (8.9%) were positive by culture. These results are a clear indication of the weakness of this diagnostic procedure. Misdiagnosing as negative about 10% of the samples represents a serious risk for the general population. Efforts have to focus in implementing more accurate diagnostic methods to reduce as much as possible the risk of spreading the disease to the susceptible population because of missing cases.

The 17% of dairy animals with gross lesions at slaughter found in this study are similar to the 16% prevalence for dairy cattle in the field officially reported in México.<sup>2</sup> However, these two prevalences are not to be compared since the official prevalence is based on animals reacting to

the tuberculin test in the field, and our sample of animals at slaughter is not necessarily a representative sample of the general population of dairy animals in México. Nevertheless, the closeness in the prevalence rates may be an indication that sampling gross lesions at slaughter may be a useful approach to estimate the prevalence of TB in the field.

From 102 gross lesions analyzed by histopathology 95% (n=97) were compatible with TB and presented acid-fast bacilli. Seventy nine percent (n=81) were positive to isolation of *M. bovis*. These findings indicate that in regions with high prevalence, diagnosis of TB through recognition of gross lesions at slaughter can be an accurate and reliable method of detecting and eliminating sources of infection. For this to work, the problem of animal identification to link affected animals to origin needs to be solved.

As in many parts of the world, the number of cases of human tuberculosis in México has increased considerably in the last five years. Low social status and concurrent immuno-compromising diseases seem to be the main risk factors. The increase in the number of cases of human TB may or may not be related to the prevalence of the disease in animals. It was the authors intention to look at the relationship between the prevalence rate in human and cattle in a kind of a ecological-study setting, unfortunately, there was not enough information about cattle TB to make any comparison feasible. It would have been interesting to see if regions with more cases of TB in cattle had also more human cases.

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#### REFERENCES

1. Committee on bovine tuberculosis, Board of agriculture. 1994. National Academy Press, 2101 Constitution Avenue, N.W. Washington, D.C. 20418.
2. CONASA. 1997. Reporte Anual del Consejo Nacional de Sanidad Animal. Centro Médico Nacional. México, D. F.
3. Corner, L. A. 1994. Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. *Vet. Microbiol.* 40:53-63.
4. Corner, L. A., A. Melville, L. McCubbin, K. J. Small, B. S. McCormicks, P. R. Wood, and J. S. Rothel. 1990. Efficiency of inspection procedures for the detection of tuberculous lesions in cattle. *Aust. Vet. J.* 67:389-392.
5. Daborn, C. J., and J. M. Grange. 1993. HIV/AIDS and its implications for the control of animal tuberculosis. *Br. Vet. J.* 149:405-417.
6. Dankner, W. M., N. J. Waecker, M. A. Essey, K Moser, M. Thomson and C. E. Davis. 1993. *Mycobacterium bovis* infections in San Diego: a clinico-epidemiologic study of 73 patients and a historical review of a forgotten pathogen. *Medicine (Baltimore)* 72:11-37.
7. Dolin, P. J., M. C. Raviglione, A. Kochi. 1994. Global tuberculosis incidence and mortality during 1990-2000. *Bull. W. H. O.* 72:213-220.
8. Epidemiología. 1997. Enfermedades Respiratorias. Sistema único de información para la vigilancia epidemiológica. Semana 25, 15 al 21 de junio. México.
9. Ferebee, S. H. 1970. Controlled chemoprophylaxis studies in tuberculosis: a general review. *Adv. Tuberculosis Res.* 17:28-106.
10. Morbidity and mortality weekly report. 1995. Essential components of a tuberculosis prevention and control program: recommendations of the advisory council for the elimination of tuberculosis. 44:1-18.
11. Panamerican Health Organization. 1991. Health conditions in the Americas, 1990, vol. 1. Scientific Publication No. 524. Pan-American Health Organization, Washington, D.C.
12. Payeur, J. B., J. L. Jarnagi, J. J. Marquardt, L. A. Schaper, B. M. Martin. 1992. Laboratory Methods in Veterinary Mycobacteriology for isolation and identification of mycobacteria. NVSL-USDA:APHIS, Ames, Iowa.
13. Sutherland, I. and P. M. Fayers. 1975. The association of the risk of infection with age. *Bull. Int. Union Tuberculosis.* 50:71-81.
14. Vynnycky, E., and P. E. M. Fine. 1997. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. *Epidemiol. Infect.* 119:183-201.
15. World Health Organization. 1996. Groups at risk: WHO report on the tuberculosis epidemic. Geneva, Switzerland: World Health Organization.