

VIRULENCE IN THE GUINEA PIG OF *M. TUBERCULOSIS* STRAINS ISOLATED FROM UNTREATED PATIENTS IN NINE AMERICAN COUNTRIES¹

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Experiences in southern India (7) have suggested that low-virulence strains of Mycobacterium tuberculosis could be producing immunological effects that interfere with the protective effects of BCG vaccine. The study reported here sought to determine the prevalence of similar low-virulence strains in nine countries of the Americas. Its results suggest that the prevalence of such strains in those countries is relatively low.

Introduction

Studies by Frimodt-Moeller (1), confirmed by Mitchison et al. (2, 3), have shown that approximately 75 per cent of the *M. tuberculosis* strains isolated from untreated patients in South India showed less virulence in the guinea pig and greater sensitivity to hydrogen peroxide than did *M. tuberculosis* strains isolated in England (4).

Renewed interest has been focused on these findings as a result of a study of BCG vaccination results conducted in South India, under

the auspices of the Indian Council for Medical Research in collaboration with WHO and the United States Centers for Disease Control (CDC). This study found that the protection supposedly conferred against bacillary pulmonary tuberculosis by BCG vaccination could not be demonstrated (5).

Infection of a high percentage of the population in this region of India with the aforementioned low-virulence *M. tuberculosis* variant could help to explain the results observed. That is because low-virulence bacilli, though infectious, rarely cause disease soon after they

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infect a person. However, such low-virulence strains can induce an immune response competing with that induced by BCG vaccination, and they are capable of causing disease later through endogenous reactivation. Therefore, their presence could partly explain the observed failure of BCG to confer protection (6).¹³

In the Americas, studies on the virulence in guinea pigs of *M. tuberculosis* strains isolated from untreated patients were carried out in Buenos Aires, Argentina, in 1966 (8) and Bahía, Brazil, in 1970 (9). No attenuated virulence was observed in either study. The present collaborative study was designed to analyze many sample *M. tuberculosis* strains, from various countries of the Americas, in order to assess their virulence in guinea pigs and their susceptibility to hydrogen peroxide, with a view to establishing whether low-virulence bacterial variants in these countries could interfere with the protective effect of BCG vaccination.

Materials and Methods

Samples of 159 *M. tuberculosis* strains isolated from patients before treatment in nine countries of the Americas¹⁴ were cultured on Lowenstein Jensen medium and sent to the Pan American Zoonosis Center. These strains were tested for niacin production and catalase activity at an ambient temperature of 68°C (10); they were also examined for pigmen-

tion, isoniazid (INH) sensitivity, and hydrogen peroxide sensitivity, as well as for virulence in guinea pigs.

The sensitivity to hydrogen peroxide was determined according to the method described by Narayanan Nair et al. (4), wherein the results are expressed as the percentage of bacilli surviving exposure to a 0.02 per cent hydrogen peroxide solution for 90 minutes.

The virulence of the strains in guinea pigs was determined according to the method described by Mitchison et al. (2, 3). Specifically, each strain was inoculated by the subcutaneous route into four guinea pigs from CEPANZO's breeding stock. These animals, all short-haired albino males weighing 350-400 g, each received 1 mg of bacillary mass. The number of bacilli inoculated was controlled by enumeration of colonies growing in subcultures.

Of the four animals inoculated with each strain, two were sacrificed six weeks after inoculation and two were sacrificed 12 weeks after inoculation, if they had not died earlier.¹⁵ A postmortem examination was then performed, and the degree to which the disease was evident in the spleen, liver, lungs, inoculation site, and associated lymph nodes was scored on a scale of 0 to 100.

This score, divided by the subject animal's survival time in days (regardless of whether it died or was sacrificed), constituted the strain's "index of virulence." This index was converted to a "root index," following the procedure of Mitchison et al. (2, 3), by taking its square root. The same experiments and procedures used to derive this value for the test strains were also performed simultaneously with the standard *M. tuberculosis* strains H₃₇Rv (virulent), H₃₇Ra (avirulent), and Indian 79157, TMC 120 (attenuated virulence in guinea pigs).

¹³ "Whereas BCG vaccination may protect uninfected persons against primary and evolutive tuberculosis, as well as against endogenous reinfection, it cannot be expected to protect uninfected persons if their eventual disease were of the exogenous reinfection type; for at the time of reinfection the level of immunity would be that derived from the primary infection, whether BCG had been given before this or not, so that controls and vaccinated subjects would have the same risk of disease from endogenous reinfection." (7).

¹⁴ Argentina, Brazil, Canada, Chile, Colombia, Costa Rica, Ecuador, Mexico, and Venezuela.

¹⁵ All the guinea pigs inoculated with one Canadian strain died within six weeks of inoculation. Appropriate numbers of the guinea pigs inoculated with the other strains either died within the allotted 0-6 and 7-12 week periods or were sacrificed at the end of 6 and 12 weeks.

Results

Identification Tests

The results of the niacin, catalase, pigmentation, and INH sensitivity tests indicated that all but seven of the 159 strains tested were found to consist of catalase-positive *M. tuberculosis* sensitive to INH. Of the 28 Mexican strains, one was found to consist of other mycobacteria belonging to the *M. intracellulare-scrofulaceum* complex and four were found to be *M. tuberculosis* strains that were weakly catalase-positive but resistant to INH. Two other *M. tuberculosis* test strains also proved to be INH-resistant, these being a weakly catalase-positive strain from Costa Rica and a catalase-negative strain from Venezuela.

Sensitivity to Hydrogen Peroxide

In all but 12 cases, at least 50 per cent of the bacilli exposed to a 0.02 per cent hydrogen peroxide solution for 90 minutes survived. The exceptionally sensitive strains included the four INH-resistant strains from Mexico, the INH-resistant strain from Costa Rica, and seven INH-sensitive strains (two from Mexi-

co, four from Costa Rica, and one from Chile). The percentages of the INH-resistant strains surviving the exposure ranged from 10 to 30 per cent, while the percentages of the INH-sensitive strains surviving ranged from 15 to 40 per cent.

Virulence in Guinea Pigs

The root indices of virulence obtained for the 152 catalase-positive INH-sensitive *M. tuberculosis* strains tested are shown in Tables 1 and 2. Most of these strains attained index values at or above the 0.8 level indicating high virulence in guinea pigs.

The results obtained with these 152 strains, grouped by country of origin, in terms of average postmortem scores and guinea pig survival times are shown in Figure 1. In general, low-virulence strains would not be expected to attain markedly higher scores at 12 weeks than at six. Therefore, the positive slope of the mean values charted makes the overall behavior of the tested strains look very similar to the behavior of high-virulence strains (with root index values of 0.8 or higher) described by Mitchison et al. (3).

Figure 2 charts relationship between the

Table 1. Root indices of virulence derived for the 83 INH-sensitive *M. tuberculosis* test strains isolated from patients in the participating countries of South America.

Approximate root index of virulence	South American test strains, by country of origin											
	Argentina		Brazil		Colombia		Chile		Ecuador		Venezuela	
	Strains at 6 weeks	Strains at 12 weeks	Strains at 6 weeks	Strains at 12 weeks	Strains at 6 weeks	Strains at 12 weeks	Strains at 6 weeks	Strains at 12 weeks	Strains at 6 weeks	Strains at 12 weeks	Strains at 6 weeks	Strains at 12 weeks
0.4	—	—	—	—	—	—	—	1	—	—	—	—
0.6	—	—	—	—	—	—	—	—	—	—	—	—
0.8	—	3	—	7	—	3	—	1	—	2	—	7
1.0	2	9	5	10	1	3	1	13	1	6	1	3
1.2	12	5	11	—	4	—	11	1	6	1	5	—
1.4	3	—	2	1	1	—	4	—	2	—	11	7
Total no. of cultures	17	17	18	18	6	6	16	16	9	9	17	17
Average root index	1.31	1.10	1.26	1.04	1.30	1.02	1.31	1.05	1.33	1.07	1.39	1.15
Standard deviation	± 0.11	± 0.08	± 0.12	± 0.14	± 0.09	± 0.13	± 0.10	± 0.16	± 0.12	± 0.11	± 0.13	± 0.20

Figure 1. Results with 152 *M. tuberculosis* test strains, grouped by country of origin, in terms of average postmortem scores and test guinea pig survival times. Markedly positive slopes indicate high virulence.

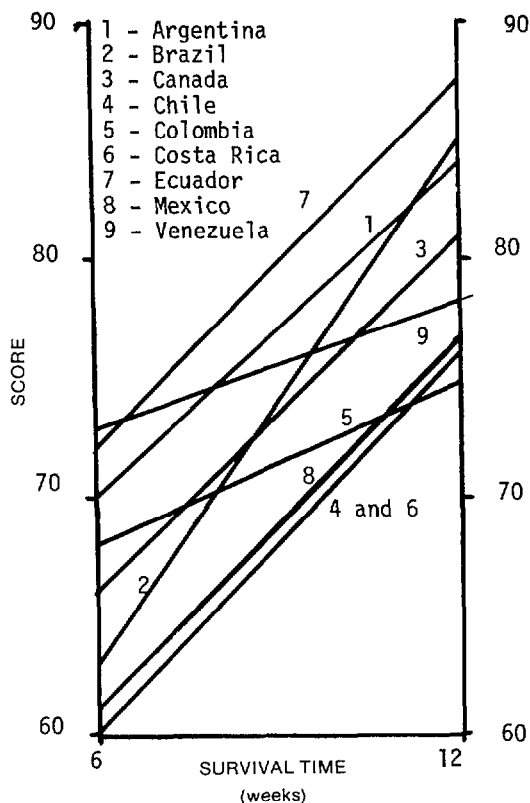


Figure 2. Results obtained, in terms of postmortem scores and guinea pig survival times, with the three standard *M. tuberculosis* strains tested and with nine test strains whose root indices showed relatively low virulence.

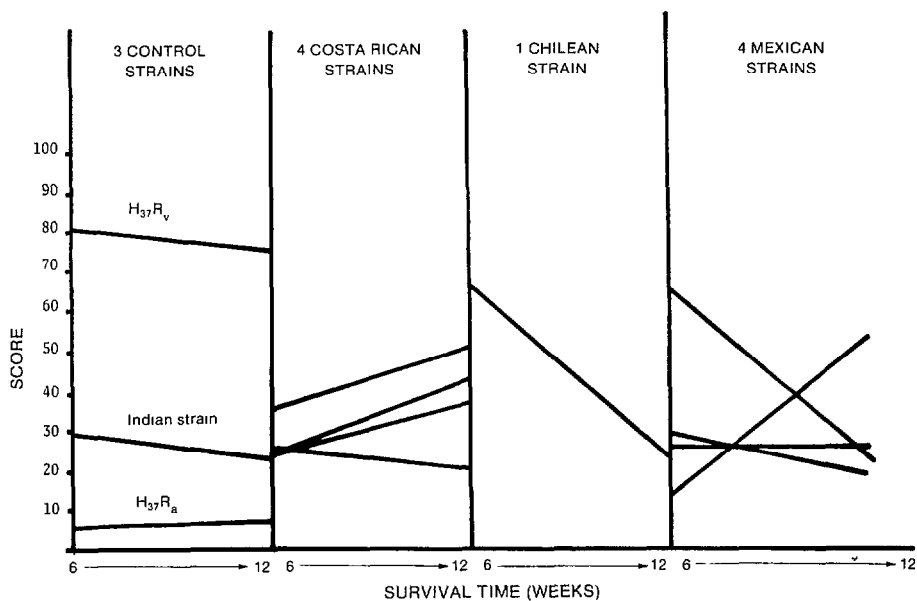


Table 2. Root indices of virulence derived for the 69 INH-sensitive *M. tuberculosis* test strains isolated from patients in the participating countries of North and Central America.

Approximate root index of virulence	Central and North American test strains, by country of origin					
	Canada		Costa Rica		Mexico	
	Strains at 6 weeks	Strains at 12 weeks	Strains at 6 weeks	Strains at 12 weeks	Strains at 6 weeks	Strains at 12 weeks
0.4	—	—	—	—	1	2
0.6	—	—	2	4	1	2
0.8	—	1	2	5	1	3
1.0	2	2	6	14	4	13
1.2	7	9	17	10	12	3
1.4	4	—	6	—	4	—
Total no. of cultures	13	12 ^a	33	33	23	23
Average root index	1.34	1.26	1.23	1.09	1.25	1.00
Standard deviation	± 0.12	± 0.15	± 0.21	± 0.20	± 0.22	± 0.22

^aAll four guinea pigs inoculated with one of the Canadian strains died within six weeks of inoculation. That strain has been eliminated from the "12 week" column because its virulence beyond the six-week period could not be tested.

postmortem scores and guinea pig survival times in tests with the three standard strains and with the INH-sensitive strains showing the lowest virulence. Most of the low-virulence strains (all four of those from Costa Rica, the one Chilean strain, and two of the four from Mexico) also showed unusual sensitivity to hydrogen peroxide.

Conclusions

In general, the virulence values obtained appear comparable to those previously observed in British *M. tuberculosis* strains, taking into account the differences to be expected in experiments carried out by different researchers using animals with different origins.

Nevertheless, a number of individual strains demonstrated attenuated virulence in guinea pigs (root index values ranging from 0.40 to 0.79). These nine strains constituted 5.9 per cent of the overall sample of 152 INH-sensitive strains, 17.4 per cent of the 23 INH-sensitive Mexican strains, 12.1 per cent of the 33 INH-sensitive Costa Rican strains, and 6.3 per cent of the 16 Chilean strains. These percentages of strains exhibiting low virulence are all relatively small compared to the 75 per cent found to exhibit low virulence in South India (1, 2, 3). These results suggest that so far as the countries included in the study are concerned, the current probability of low-virulence *M. tuberculosis* strains competing immunologically with BCG vaccine are very slight.

SUMMARY

Failure to demonstrate the effectiveness of BCG vaccination in South India, together with a high prevalence of low-virulence *M. tuberculosis* strains in that area, has suggested that these low-virulence

variants could be producing immunological effects that interfere with those ordinarily produced by BCG (7). The study reported here was designed to test *M. tuberculosis* strains isolated from untreated

patients in nine countries of the Americas in order to assess the prevalence of low-virulence strains in those countries.

For this purpose, 159 strains were tested for virulence in guinea pigs and sensitivity to hydrogen peroxide (another characteristic of the low-virulence strains from India), and also for INH sensitivity, catalase activity, niacin production, and pigmentation.

The results indicated that the vast majority of the 152 INH-sensitive strains tested were normally

virulent in guinea pigs. Nine of these strains (17.4 per cent of those from Mexico, 12.1 per cent of those from Costa Rica, and 6.3 per cent of those from Chile) did exhibit low virulence in guinea pigs. However, in all cases the percentage of low-virulence strains was far less than that prevailing in southern India, suggesting that so far as the study countries are concerned, the current likelihood of low-virulence *M. tuberculosis* strains competing immunologically with BCG is very slight.

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