

# Level of serum antibodies to mycobacterial antigens in healthy Czechs\*

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*Levels of IgG antibodies to Mycobacterium bovis BCG and M. avium antigens were examined by enzyme-linked immunosorbent assay (ELISA) in samples of sera from 898 healthy adults aged >40 years and 1170 children aged 1–6 years, selected at random in 10 districts of Czechoslovakia. The median antibody titre to M. bovis BCG in adults ranged from 1:28 to 1:161.7 and that in children from 0 to 1:50. The mean titres of M. avium antigen ranged from 1:2 to 1:21 in adults and from 0 to 1:8 in children.*

The study determined the levels of serum antibodies to two taxonomically distinct mycobacterial antigens among a sample of healthy children and adults selected at random from 10 districts in Czechoslovakia. The data obtained may contribute to understanding the current serum antibody responses to the mycobacteria (both pathogenic and opportune) that are circulating in these districts, and could help to establish the cut-off values between seropositivity and seronegativity for the tests used.

The study was initiated in 1987. In children the results were expected to assess the overall effect of BCG vaccination at birth and to trace the transmission of infection by tuberculous and nontuberculous mycobacterial pathogens among unvaccinated children. The sample of healthy adults over 40 years of age was chosen to detect the geographical distribution of humoral reactivity to both sets of mycobacterial antigens.

The study had a prospective design and it is intended to continue it by examining about 700 samples of sera every year from individuals in the above-mentioned age categories, chosen from randomly selected districts in Czechoslovakia. In areas where BCG vaccination at birth has been discontinued, the study may help to trace the transmission of tuberculous and nontuberculous mycobacteria from human sources and environmental reservoirs.

## Materials and methods

**Source of the sera.** Samples of sera were obtained from the Serum Bank, Institute of Hygiene and Epidemiology, Prague, where sera from healthy individuals are collected for multipurpose serological surveys (3). Altogether, 898 sera from adults over 40 years of age and 1170 sera from children aged 1–6 years were examined in the study. The sera were selected at random, and the donors lived in 10 randomly selected Czech districts.

Testing antigens were prepared by disintegrating *Mycobacterium bovis* BCG (Soviet vaccine strain 198) and *M. avium* (strain ATCC 15 769) using a technique that has been described in detail elsewhere (2).

**Enzyme-linked immunosorbent assay (ELISA).** Microtitration plates were coated with antigens diluted 1:1000 from a stock solution containing 1 mg of protein per ml. Sera at dilutions of 1:40 to 1:320 were assayed by ELISA, as described previously (1, 2). The assay for IgG antibodies was performed using SwAHuPx/IgG conjugate (USOL, Prague); the chromogen substrate 5-aminosalicylic acid with 0.005% (w/v) hydrogen peroxide was used to visualize the reaction, and the results were expressed as absorbances, which were measured with an automated ELISA reader at  $\lambda = 450$  nm.

**Statistical evaluation of the results.** The results were evaluated statistically to establish the degree of immune responses for mycobacteria and their differences according to area. A model for the dependency of the absorbance  $a$  on  $c$  (the logarithm of the antibody concentration) of the type

$$a = \exp [d + bc],$$

where  $d$  and  $b$  are unknown constants, was used (1, 2).

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We estimated values of  $d$ ,  $b$ , and  $c_{0.3}$  for each person ( $c_{0.3}$  = predicted log (concentration for  $a = 0.3$ )) by linearizing the regression function. The concentration corresponding to  $c_{0.3}$  was termed the critical titre. A dispersion analysis was carried out on these data using a classical  $F$  test; if the results of the dispersion analysis were significant, a Duncan test was then used (1, 2).

## Results

The lowest median antibody titre to the BCG antigen in adults was in Rychnov district (1:25), with the median titres in the remaining districts being greater than 1:28 (maximum titre, 1:161.7 in Náchod district). The median antibody titres in children were significantly lower than those in adults, and ranged from 0 to 1:50 (Table 1).

The highest antibody titre to *M. avium* antigen in adults was found in Náchod district (1:21.2), and, in children, in Nymburk district (1:8.2) (Table 1).

For a group of 39 unvaccinated children from Rakovník district (where the obligatory BCG vaccination of newborns was discontinued in 1986), the median value of titres to both antigens was zero. However, four children in this group produced antibodies to the BCG antigen at titres ranging from 1:16 to 1:62, and to *M. avium* antigen from 1:8 to 1:30.

## Discussion

For the detection of serum antibodies, we used the following antigens in parallel: the antigen of *M. bovis* BCG, as a carrier of common antigenic determinants of the *M. tuberculosis* complex; and the *M. avium* antigen, whose role was to demonstrate the

presence of antibodies to nontuberculous environmental mycobacteria.

In adults, antibody titres to BCG antigen that exceeded 1:100 were recorded only in two districts. The titres in the remaining districts ranged from 1:25 to 1:98 and were close to the values found in control blood donors in a serodiagnostic study (1:65.2) (1), as well as being comparable to those recorded in a similarly designed study of the normal, healthy population in Yemen (1, 2). For children, the highest titres occurred in districts where the titres for adults were high. In the remaining districts the titres for children were less than 1:10 and approached the mean titre of 1:8.4 for umbilical cord blood serum of newborns in Prague (1).

In both adults and children the reactivity to *M. avium* antigen was substantially lower than that to BCG antigen. The highest titres in adults occurred in districts with the highest response to BCG antigen, probably as a result of a cross-reaction.

The study was primarily concerned with the possible use of serum antibody assays to assess the transmission of tuberculous and nontuberculous mycobacterial infections in populations. This use is based on the premise that antibodies are formed under the pressure of infectious agents but not in uninfected areas. Unlike tuberculin testing, serum antibody detection is not influenced by booster effects, does not require the presence of the examined individual, the results are objective, and serum samples can be stored for long periods. This approach, which is rather novel in tuberculosis epidemiology, should be verified in precisely defined field studies. We plan to carry out such studies on unvaccinated children and on individuals from various age groups from randomly selected geographical areas of Czechoslovakia.

Table 1: Number of individuals examined in the study and the median values of serum antibody titres against *Mycobacterium bovis* BCG and *M. avium* in healthy adults and children in 10 districts in Czechoslovakia

District	n	Adults		n	Children	
		<i>M. bovis</i> BCG titre	<i>M. avium</i> titre		<i>M. bovis</i> BCG titre	<i>M. avium</i> titre
Beroun (BE)	97	52.2	16.8	117	6.8	0.5
Nymburk (NB)	90	116.9	7.2	139	50.1	8.2
Rakovník (RA)	89	98.0	14.5	106	2.0	0
Náchod (NA)	89	161.7	21.2	163	19.1	2.1
Rychnov (RK)	90	25.0	2.0	118	0.8	0
Svitavy (SV)	89	28.0	7.0	119	2.0	1.0
Louny (LN)	89	49.0	5.0	90	5.0	0
Olomouc (OL)	89	80.4	20.5	119	3.8	1.3
Přerov (PR)	86	75.0	2.5	109	0	0
Prostějov (PV)	90	77.1	4.3	90	8.0	0.6

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## Résumé

### Anticorps sériques vis-à-vis des antigènes mycobactériens chez des Tchèques en bonne santé

Le but de cette étude a été d'obtenir à des fins épidémiologiques des données sur la réactivité immunitaire aux mycobactéries dans des groupes de population en bonne santé de différentes régions de la République fédérative tchèque et slovaque, d'après la mise en évidence par ELISA des anticorps sériques IgG dirigés contre les antigènes de *M. bovis* BCG (souche vaccinale soviétique 198) et de *M. avium* (souche ATCC 15 769). Les sérums aux dilutions de 1:40 à 1:320 ont été répartis en fractions de 100 µl dans des godets de microplaques sensibilisés par les antigènes, et les plaques ont été laissées à 4°C jusqu'au lendemain où elles ont été incubées avec le conjugué SWAHu IgG/Px. Pour visualiser la réaction, on a utilisé comme substrat l'acide 5-aminosalicylique. L'examen a porté sur 898 sérums d'adultes en bonne santé âgés de plus de 40 ans et 1170 sérums d'enfants âgés de 1 à 6 ans provenant de 10 districts.

Les médianes des titres d'anticorps anti-*M. bovis* BCG variaient entre 1:25 et 1:161,7 pour les adultes et entre 0 et 1:50 pour les enfants.

Les titres critiques d'anticorps anti-*M. avium* étaient de 1:20 à 1:21 chez les adultes et de 0 à 1:8 chez les enfants.

Sur 39 enfants non vaccinés du district de Rakovník, 35 avaient une médiane des titres d'anticorps vis-à-vis des deux antigènes testés égale à 0 et chez les quatre autres on a mis en évidence des anticorps aux titres de 1:16 à 1:62 pour l'antigène du BCG et de 1:8 à 1:30 pour celui de *M. avium*.

Nous pensons utiliser ces données pour établir la limite de la négativité du test ELISA en diagnostic et pour étudier les différences de réactivité chez les habitants de districts différant entre eux par la prévalence de la tuberculose. D'après la réactivité à l'antigène de *M. avium*, on pourrait estimer l'effet immunogène des mycobactéries non tuberculeuses. La répétition de ces tests dans différentes régions pourrait permettre d'étudier la dynamique des infections mycobactériennes dans la population après la suspension prévue de la vaccination obligatoire par le BCG.

## References

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