HEAT RESISTANCE AND VIRULENCE OF SEROVARS OF THE MYCOBACTERIUM AVIUM COMPLEX

M. PAVLAS
Veterinary Research Institute, 621 32 Brno

Received June 19, 1997
Accepted January 15, 1998

Abstract


Heat resistance and virulence tested in chickens were investigated in 151 strains classified with the Mycobacterium avium complex. The highest differences in heat resistance between virulent and avirulent strains were found at 2-h exposure to 60 °C. Under such conditions, 92% of avirulent strains classified with Mycobacterium intracellulare serovars 4, 8, 9 (Schaeffer 1965) were resistant. On the other hand only 4.8% of virulent strains of Mycobacterium avium subsp. avium serovars 2, 3 resisted the above exposure conditions.

Mycobacterium avium complex, virulence, heat resistance

Species designated already in the past century as Bacillus tuberculosis gallinarum (Sternberg 1892), identical with Mycobacterium tuberculosis typus gallinaceus (Lehmann and Neumann 1896) and Mycobacterium tuberculosis avium (Chester 1901), are currently of greatest importance among pathogenic mycobacteria.

Mycobacterial strains with biological properties closely resembling those of M. avium subsp. avium (hereinafter M. avium) were isolated from porcine lymph nodes in the fifties of this century. These porcine strains did not induce any lesions in rabbits even after intravenous inoculation. With regard to their origin, it was recommended to classify them as a separate species Mycobacterium suis (Baumann et al. 1955). Similar strains non-virulent in chickens were isolated several years later from human patients suffering from a tuberculosis-like disease. Pneumokoniosis was identified as a predisposing factor in the patients. The strains were isolated at the Battey State Hospital, Georgia, and were designated Mycobacterium battey accordingly (Crow et al. 1957).

The very close similarity of biochemical properties did not allow the differentiation of the above mycobacterial species. On the other hand, marked virulence of M. avium in birds and other animal species was a relatively reliable characteristic for distinguishing this species from M. intracellulare.

Comparative investigations of M. avium and M. intracellulare revealed differences in surface antigens allowing, by means of agglutination test, the identification of 2 serovars of M. avium and another 15 serovars of M. intracellulare (Schaeffer 1967). So far 29 serovars of the M. avium-intracellulare complex have been identified. The serovars 1, 2 and 3 were considered to belong to M. avium pathogenic for birds. According to OIE (1996), the serovar 6 of M. intracellulare ranks with potential agents inducing avian tuberculosis. There can be no doubt that serological typification of the M. avium-intracellulare complex has become an important component epidemiological investigations. Serovar 2 is apparently the most significant of them owing to its high prevalence and virulence for poultry, other bird species and mammals, particularly swine. Serovar 3 differs from serovars 1 and 2 in its specific lipid composition and surface antigens (Marks et al. 1969). Variable virulence has been reported for serovar 1 isolated frequently from human patients. The virulence of
individual serovars of *M. avium* is often dependent on the origin of the strain. Thus, for instance, virulence was demonstrated in 20% of human and 66% of porcine strains belonging to serovar 1, but in all porcine strains, 97% of bovine strains and 80% of human strains belonging to serovar 2. Bioassays in chickens revealed virulence in 97.3% of the 37 tested avian strains of serovars 1 and 2 (Schaeffer 1967a).

Results of differentiation between the two mycobacterial species using virulence-based tests are not always unequivocal. In such tests, the result depends, among other factors, on the inoculum size, inoculation route, and species, age and sex of birds. Virulence was often demonstrated in *M. intracellulare* serovars when inadequate methods were used. *M. intracellulare* serovars 4, 5, 6, 8, and 10 are regarded as facultatively pathogenic, particularly when inoculated intravenously to chickens (Meissner 1970). The most marked differences between virulent serovars of *M. avium* and *M. intracellulare* were demonstrated after intramuscular inoculation of the tested strains at a dose of 1 mg per chicken. On the other hand, intravenous inoculation of serovar 8 at the dose of 0.1 or 1.0 mg per bird induced liver and sometimes also spleen lesions leading often to death in more than 50% of chickens (Pattyn 1967; Pavlas and Patloková 1977). A very low virulence of *M. intracellulare* serovars was also demonstrated in rabbits. Arthritic lesions developed in this species 5 to 6 months after intravenous inoculation of 1 mg of such cultures (Kováčs 1962).

Available data show that differences between *M. avium* and *M. intracellulare* consist rather in the degree of virulence than in pathogenicity. Considering the within-serovar differences in virulence, the oral inoculation recommended by several authors for biological tests should be regarded as a rather rough or pilot method. More accurate results could perhaps be obtained in tests using macrophage cultures (Coster and Engel 1970).

Some authors have recommended the use growth potential at different temperatures as a characteristic allowing the differentiation between *M. avium* and *M. intracellulare* (Cowan and Steel 1965). Another characteristic for the assessment of virulence of individual serovars of the *M. avium* complex might be the higher heat resistance of *M. intracellulare* as determined by the capillary method. Investigations of heat resistance of pathogenic and facultatively pathogenic atypical mycobacteria have demonstrated marked differences in the potential to survive at 60 °C (Pavlas 1989). The objective of our experiments was to assess heat resistance of *M. avium* and *M. intracellulare* and to use contingent differences as a criterion for the differentiation between virulent and avirulent serovars of the *M. avium* complex.

**Materials and Methods**

Experience and knowledge gained during our earlier investigations of heat resistance by means of the capillary method were used and a more simple method of assessment of heat resistance in Liquid Serum-Supplemented Medium for the Culture of Mycobacteria (SEVAC, Prague) heated in a water bath to 60 °C immediately after the inoculation of the tested strain was chosen. One drop (0.05 ml) of a suspension of a 4- to 6-week-old culture of mycobacteria was inoculated into 5 ml of the medium.

A set of 151 strains of the *M. avium* complex, including 63 strains inducing generalized tuberculosis after intramuscular inoculation in chickens and 88 strains inducing only local lesions at the inoculation site, was tested. The strains were isolated from lymph nodes or parenchymatous organs of swine in herds showing positive reactions to avian tuberculin. By means of agglutination test with sera of rabbits immunized with reference antigens (Schaeffer 1965), most of the strains avirulent for poultry were identified as serovars 4, 8 or 9 (some of them reacting also with antiserum to serovar 1), while all the strains virulent for poultry were identified as serovars 2 or 3. A uniform dose of 1 mg of a 4- to 6-week-old mycobacterial culture homogenized in 0.5 ml of sterile physiological saline was administered into pectoral muscles.
Test tubes containing mycobacterial cultures in the liquid medium were exposed in a water bath to 60 °C (100 °C) for 30, 60, 120, or 240 min. A fifth tube containing an unexposed culture was used as the control. After the exposure, the test tubes were cooled with running water and put into a thermostat. Growth was read after 2, 4 and 8 weeks of incubation at 37 °C.

Results

A significant difference in heat resistance at 60 °C was found between chicken-virulent and chicken-avirulent strains of the \textit{M. avium} complex. Three (4.8\%) of the 63 virulent strains, belonging to \textit{M. avium} serovars 2 or 3, and 81 (92\%) of the 88 strains of avirulent \textit{M. intracellulare} belonging to serovars 4, 8, or 9 propagated after a 120-min exposure to 60 °C (Table 1). No significant differences in heat resistance were found among the above \textit{M. intracellulare} serovars.

The results demonstrate evident differences in heat resistance between virulent and avirulent serovars of the \textit{M. avium} complex that might be used in the assessment of biological properties of atypical mycobacteria.

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Virulence</th>
<th>Number of mycobacterial strains*</th>
<th>Growth after exposure to 60 °C for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>4, 8, 9</td>
<td>I</td>
<td>88/0</td>
<td>88/0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100/0)</td>
<td>(100/0)</td>
</tr>
<tr>
<td>2, 3</td>
<td>II</td>
<td>63/0</td>
<td>63/0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100/0)</td>
<td>(100/0)</td>
</tr>
</tbody>
</table>

I – only local lesions at the inoculation site
II – lesions at the inoculation site and in parenchymatous organs
* Numbers (\%) of resistant and sensitive strains are given in numerator and denominator, respectively.

Discussion

Our experiments were based on published data on growth properties of \textit{M. avium} and \textit{M. intracellulare} at 22 and 45 °C (Cow an and Steel 1965; Pattyn and Portaels 1972) and on heat resistance of atypical mycobacteria at 60, 65, 70, and 75 °C. The exposure to 60 °C, found in our previous studies as the most suitable, was chosen for heat resistance tests of virulent and avirulent serovars of the \textit{M. avium} complex.

Many characteristics common to \textit{M. avium} and \textit{M. intracellulare} have been revealed by means of techniques of molecular biology (Wayne et al. 1993) and it has been suggested to include the two mycobacterial species into the \textit{Mycobacterium avium} complex. Irrespective of the further development in the taxonomy of \textit{M. avium} and \textit{M. intracellulare}, there exist differences in virulence and other biological properties among individual serovars of the two species that should be considered in the assessment of their health significance for individual animal species.
Termorezistence a virulence serovárů Mycobacterium avium complex

Byla ověřována termorezistence 151 kmenů Mycobacterium avium complex ve srovnání s jejich virulencí hodnocenou pomocí biologických pokusů na kuřatech. Největší rozdíly v termorezistenci kmenů virulentních a avirulentních byly zjištěny při teplotě 60 °C a expozici 2 hodiny. Při této teplotě a expozici bylo rezistentněch 92 % kmenů avirulentních zařazených podle serotypizace (Schaeffer 1965) do skupiny Mycobacterium intracellulare, serovar 4, 8, 9. Naproti tomu kmenů Mycobacterium avium subsp. avium (dále M. avium), serovar 2, 3 bylo odolných ke štěru uvedené teplotě a expozici pouze 4,8 %.

References

MEISSNER, G. 1970: Serotyp-Beitstillungen zu Mykobakterien der aviären Gruppe. Ihre mikrobiologische und epidemiologische Bedeutung. Symposium o atypických mykobakteriích, KHS Plzeň, pp. 72-78

Address for correspondence
Doc. MVDr. M. Pavlas, DrSc.
State Veterinary Institute
Palackého 174
612 00 Brno
Czech Republic
Phone: 420 5 41 32 12 29