Use of the Immunofluorescence Method in an Epidemic Focus of Tularaemia

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ABSTRACT

Four hares found dead and 32 animals (small rodents, weasels, deer) caught in a focus of tularaemia were examined by the immunofluorescence method. In full agreement with the biological test, the use of fluorescent antibodies showed the presence of Pasteurella tularensis in the hares, even when the organs were massively contaminated with other bacteria, making cultivation tests quite impossible. In the other animals, all the test methods gave negative results. Tests of sera from 149 convalescent patients and 59 control subjects showed complete agreement (positivity or negativity) between the indirect immunofluorescence method and the agglutination reaction. The question of the titres found in the indirect immunofluorescence reaction are discussed. The first results with the use of fluorescent antibodies in practice thus confirm that this is a suitable method for examining animals in epizootic foci and for detecting specific antibodies in human serum.

The fluorescent antibody method was found completely satisfactory, under experimental conditions, for identifying Pasteurella tularensis cultures and for detecting this pathogen in the organs of infected animals (Franěk & Procházká, 1965; Franěk, 1965). It was therefore decided to test it in practice.

An opportunity was provided by a tularaemia epidemic in West Bohemia at the beginning of 1964.* The suitability of fluorescent antibodies for demonstrating Pasteurella tularensis directly in naturally infected animals was studied (together with the optimal conditions of collecting and treating the material) and the effectiveness of the indirect immunofluorescence method for rapid demonstration of specific antibodies in the serum of patients and convalescent was determined.

MATERIALS AND METHODS

When testing material by the immunofluorescence technique, 0.15% conjugate prepared from rabbit serum E-9/64 and characterized in detail in a previous paper (Franěk & Procházká, 1965) was used. The preparations were further stained with albumin conjugated with lissamine rhodamine B 200, by a method already described (Franěk, 1965). For the indirect immunofluorescence reaction, rabbit anti-human fluorescein-conjugated globulin supplied by Microbiological Associates, Bethesda, U.S.A. (batch 21849) was used.

Hares sent for examination were treated as follows: spleen, liver and bone marrow impressions were done and suspensions were prepared by homogenizing 1 g. of each organ in 1 ml. saline. The serially diluted homogenates were then admin-

*) The natural focus was investigated by Dr. D. Lím, of the Institute of Epidemiology and Microbiology, Prague and members of the army sanitary corps.
istered to mice, inoculated on blood agar containing sodium thioglycolate and used for the preparation of smears for the fluorescent antibodies reaction.

The spleens of small rodents caught in the focus (after the epidemic) were used for impression smears; bone marrow homogenate was used for smears and for infecting white mice.

The biological test was carried out with white mice weighing 18—20 g., which were challenged subcutaneously with 0.1 ml. of the appropriate material.

Positive human sera were chosen from material examined by the Microbiology Department of the District Station of Hygiene and Epidemiology in Žatec (136 sera) and in the Serological Laboratory of the Regional Station of Hygiene and Epidemiology, Ústí nad Labem (15 sera). Control sera were obtained from the Diagnostic Serological Laboratory of the Military Institute of Hygiene, Epidemiology and Microbiology, Prague; they comprised the sera of patients examined in different departments of the Central Military Hospital, Prague, or of healthy subjects. The sera of nine patients with brucellosis were obtained from the Clinical Laboratory, Bulovka Hospital, Prague. *Pasteurella tularensis* strain isolated from hare No. 1 (temporarily denoted as *Pasteurella tularensis* P-64) was used as the antigen for immunofluorescence and agglutination reactions.

**RESULTS**

Results of comparative tests of the organs of dead hares. Altogether four hares were examined, three of which were found in the focus itself; the fourth was found later in the marginal zone.

Hare No. 1 had been dead about 24—48 hours and No. 2 about 5—7 days. Because of the cold weather (February 1964), the organs had not undergone serious decomposition. Hare No. 3 was found in the focus in March 1964. It was in an advanced state of decomposition and only the bone marrow could be used for laboratory tests. Hare No. 4 was caught about 61/2 miles away from the focus, in May 1964, i.e. three months after the epidemic. It was obviously ill

<table>
<thead>
<tr>
<th>Hare No.</th>
<th>Organ</th>
<th>Death of mice (days after infection)</th>
<th>Numerator: total number of dead mice</th>
<th>Denominator: number of mice in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spleen Bone marrow</td>
<td>3/7 6/7 7/7</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Spleen Bone marrow</td>
<td></td>
<td>2/6 6/6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Bone marrow</td>
<td></td>
<td>2/4 4/4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Spleen Bone marrow</td>
<td>2/4 4/4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Demonstration of *Pasteurella tularensis* in organs of dead hares (biological test)