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54. THE FIRST EPIDEMY OF TULAREMIA IN FR YUGOSLAVIA

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INTRODUCTION:

Tularemia is zoonotic disease caused by *Francisella tularensis*. The reservoirs of disease are the mammals of the genera *Lagomorpha* and *Rodentia* and vectors are ixodid ticks and other haematophagous insects. Tularemia is predominantly a disease of the northern hemisphere, and the form of the disease depends on the route of entry of *F.tularensis* in the organism. The ulceroglandular, tonsillopharyngeal, gastrointestinal and pulmonary form of disease occur most often. *F.tularensis* can be used as a weapon in biological warfare. Immunodiagnostic procedures, based on detection of specific antibodies on *F.tularensis* in sera are most often used in diagnosis of tularemia. Although FR Yugoslavia is an endemic region, the first epidemic of tularemia in our country broke out in 1998, in the region of mountain Rtanj, near Sokobanja. In that period thirty-eight people, between 7 and 77 years, had the disease. The most often appeared tonsillopharyngeal form and rarely ulceroglandular form of tularemia. The epidemic lasted from 1999 through 2000, in the wider region of Sokobanja, including villages near Pirot and Aleksinac. During 1999. and 2000. tularemia appeared in the southern Serbian province, Kosovo and Metohia, but unfortunately we have no evidence nor any data about disease from that region. For the first time in FR Yugoslavia, in the beginning of 1999, *F.tularensis* was isolated from dead individuals of the genus *Apodemus* in the infected region.

This work is devoted to biochemical, genetic and immunochemical characterization of that isolate and the use of our isolate as antigen for preparation and standardization of homemade immunodiagnostic procedures, such as agglutination, immunofluorescence and immunoenzyme tests.

MATERIALS AND METHODS:

We developed the microagglutination test using the reference strain Schu (S84) of *F.tularensis tularensis*. This was used for examination of the sera of people from infected region. The biological assay on experimental mice was used for isolation of *F.tularensis* from dead animals of the genus *Apodemus* from infected region. *F.tularensis* was cultivated on the Francis medium enriched with cysteine. The virulence of isolated strain of *F.tularensis*, its erythromycin sensitivity, the metabolism of glycerol, glucose and cytrulin-ureidase activity were examined. rRNA hybridization was used for confirmation of the isolate and for its genetic typing. SDS-PAGE was used for immunochemical characterization of the isolate and its comparison to other *F.tularensis* strains. The domestic isolate of *F.tularensis* was used as antigen for preparation and standardization of homemade immunodiagnostic procedures, such as agglutination test, immunofluorescence test.

RESULTS AND DISCUSSIONS:

Examination of the sera of people from contaminated region by the microagglutination test showed that 38 persons (between 7-77 years) gave positive reaction. All of them had typical clinical signs of tonsillopharyngeal tularemia, except two who had the ulceroglandular form of disease. Agglutination titers ranged between 1:80-1:2560.

Using biological assays from dead individuals of the genus *Apodemus* from the contaminated region, *F.tularensis* was isolated at the Institute for Microbiology, Military Medical Academy, Belgrade (Lako B., Ristanovic E.) in the beginning of 1999. It was the

first isolate of *F.tularensis* in FR Yugoslavia. Although our country is in the endemic region for tularemia, there has not been an epidemic of this disease in FR Yugoslavia before, and the disease appears only rarely, so we had not isolated strain of *F.tularensis*. Until now, for diagnosis of tularemia in our country, we used only agglutination tests made by use of reference strain Schu (S84) of *F.tularensis* as the antigen. The domestic isolate of *F.tularensis* is high virulent for experimental animals, erythromycin sensitive, it metabolizes glucose and it has no cytrulin-ureidase activity. It is cultivated and stored on Francis media enriched with cysteine, which is necessary for growth of *F.tularensis*. Hybridization with fluorescent-labeled probes specific for rRNA of *F.tularensis* confirmed that isolated strain belongs to *Francisella tularensis* subsp. *palaeartica*. The results of SDS-PAGE show that both strains (isolated and standard laboratory strain) of *F.tularensis* have protein bands of the same electrophoretical mobility. Because of that, this method cannot be used for gene typing of *F.tularensis* strains (Fig 1.). The immunodiagnostic procedures made by use of domestic isolate of *F.tularensis*-Rtanj as the antigen are more sensitive and more specific than procedures based on use of laboratory strains of *F.tularensis*-Schu (S84).

The sensitivity of microagglutination test (MAT) made by use of isolated strain *F.tularensis*-Rtanj as the antigen is 86.36% and its specificity is 94.12%. The sensitivity and specificity of the same test (MAT) made by use of referent strain-Schu *F.tularensis* as antigen are lower, their values are 81.82% and 92.16%, respectively. The indirect immunofluorescence test (IIF) is generally more sensitive and more specific than microagglutination test. IIF test enables us to detect and determine each immunoglobulin class (not only IgM antibodies), and because of that it is better than agglutination tests. The sensitivity of IIF test using isolated strain *F.tularensis*-Rtanj, as antigen is 88.64%, and its specificity is 94.34%. The sensitivity and specificity of IIF test using the reference strain-Schu *F.tularensis* as antigen were the same. The preparation and standardization of homemade immunoenzyme (ELISA) test is in progress.

CONCLUSIONS:

The first epidemic of tularemia in FR Yugoslavia broke out at the end of 1998 in the region of mountain Rtanj, near Sokobanja. The epidemic lasted from 1999 through 2000, in the wider region of Sokobanja, including the villages near Pirot and Aleksinac. A detailed examination of these natural foci of tularemia is in progress. For the first time in our country, *F.tularensis* was isolated from dead individuals of the *Apodemus* genus. This isolate was confirmed by rRNA hybridization and identified as *F.tularensis* subsp. *palaeartica*. Immunodiagnostic procedures (agglutination and immunofluorescent) using the isolated strain *F.tularensis*-Rtanj as the antigen are more sensitive and specific than procedures using the referent strain Schu (S84). The preparation and standardization of homemade immunoenzyme (ELISA) test is in progress.

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SUMMARY:

Tularemia is zoonotic disease caused by *Francisella tularensis*. The reservoirs of disease are the mammals of geni *Lagomorpha* and *Rodentia*, and vectors are mainly ixodic ticks and other haematophagic insects. *F.tularensis* can be used as a weapon in biological warfare. Immunodiagnostic procedures, based on detection of specific antibodies on *F.tularensis* in sera are most often used in the diagnosis of tularemia.

Although FR Yugoslavia is in an endemic region, the first epidemic of tularemia in our country broke out in 1998 in the region of mountain Rtanj, near Sokobanja. In that period thirty-eight people, between 7 and 77 years, had the disease. The epidemic lasted from 1999 to 2000. The detailed examination of these natural foci of tularemia is in progress.

For the first time in FR Yugoslavia *F.tularensis* was isolated from dead individuals of the genus *Apodemus* from infected region in the beginning of 1999. This isolate was confirmed by rRNA hybridization with probes specific for genus *Francisella*, species *F.tularensis*, subs. *F.tularensis palaeartica*. The domestic isolate of *F.tularensis* is high virulent for experimental animals, erythromycin sensitive, it metabolizes glucose and it has no citrulin-ureidase activity. This isolate is used as antigen for preparation and standardisation of home-made immunodiagnostic procedures, such as agglutination (rapid- and microagglutination test), immunofluorescent (IIF) and immunoenzyme (ELISA) test.

KEYWORDS

Tularemia, *Francisella tularensis*, isolation, rRNA typisation, immunodiagnostic procedures

FIGURES:

Figure 1. SDS-PAGE proteins of *F.tularensis*



1 2 3

1-isolated strain of *F.tularensis palaeartica*-Rtanj
2-reference strain of *F.tularensis tularensis*-Schu (S84)
3-markers of molecular weight