



Tularemia

Epidemiological, clinical and diagnostic aspects

av

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Akademisk avhandling

Avhandling för medicine doktorsexamen i medicin,
som enligt beslut av rektor kommer att försvaras offentligt
fredagen den 14 mars 2008 kl. 12.30,
Wilandersalen, Universitetssjukhuset Örebro

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ABSTRACT

Tularemia is a zoonosis caused by the small, fastidious, gram-negative rod *Francisella tularensis* that appears over almost the entire Northern Hemisphere. In Sweden, tularemia has appeared mainly in restricted areas in northern parts of central Sweden.

The disease can be transmitted through several routes: direct contact with infected animals, by vectors, through contaminated food or water or through inhalation of aerosolized bacteria. Distinct clinical forms of the disease are seen, depending on the route of transmission. During the last years, tularemia has emerged in new areas in central Sweden, south of the endemic area. The emergence of tularemia in the County of Örebro prompted the investigations presented in this thesis.

We performed a case-control study, using a mailed questionnaire, to identify risk factors for acquiring tularemia in Sweden (**Paper I**). After multivariate analysis, mosquito bites and cat ownership could be associated with tularemia in all studied areas while farming appeared as a risk factor only in endemic areas.

In **Paper II**, we evaluated a PCR analysis, targeting the *tul4* gene, used on samples from primary lesions in patients with ulceroglandular tularemia. The method performed well, with a sensitivity of 78% and a specificity of 96%.

The clinical characteristics of tularemia in an emergent area in Sweden were studied (**Paper III**), using case files and a questionnaire. Of 278 cases of tularemia reported during the years 2000 to 2004, 234 had been in contact with a doctor from the Department of Infectious Diseases at Örebro University Hospital, and were thus included. The ulceroglandular form of the disease was seen in 89% of the cases, with the primary lesion, in most cases, on the lower leg. An overwhelming majority of cases occurred during late summer and early autumn, further supporting transmission by mosquitoes. Erythemas overlying the affected lymph node areas were seen in 19% of patients with forms of tularemia affecting peripheral lymph nodes. Late skin manifestations, of various appearances, were seen in 30% of the cases, predominantly in women. A raised awareness of tularemia among physicians in the county during the course of the outbreak was found, as documented by the development of shorter doctor's delay and less prescription of antibiotics inappropriate in tularemia.

Finally, we developed a simplified whole-blood lymphocyte stimulation test, as a diagnostic tool in tularemia (**Paper IV**). The level of IFN- γ , as a proxy for lymphocyte proliferation, was measured after 24-h stimulation. Additionally, a tularemia ELISA with ultra-purified LPS as the antigen was evaluated, showing a high sensitivity. The lymphocyte stimulation test, when performed on consecutive samples from subjects with ongoing tularemia was able to detect the disease earlier in the course of the disease than both the new ELISA and the tube agglutination test. Furthermore, all tularemia cases became positive in the lymphocyte stimulation test within 12 days of disease.

In conclusion, this thesis describes risk factors for acquiring tularemia as well as the clinical characteristics of the disease in Sweden. Additionally, a *Francisella* PCR analysis and a tularemia ELISA based on highly purified LPS is evaluated, and a simplified lymphocyte stimulation test, for early confirmation of the disease, is developed.

Keywords: Francisella; tularemia; epidemiology; case-control; diagnosis; PCR; clinical characteristics; immunology; ELISA; lymphocyte stimulation

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