Case Report

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Septic pneumonic tularaemia caused by *Francisella tularensis* subsp. *holarctica* biovar II

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This case of pneumonic tularaemia elucidates two aspects: it is believed to be the first documented case of bacteraemia caused by *Francisella tularensis* subsp. *holarctica* biovar II; furthermore, it illustrates the remission of septic pneumonic tularaemia without appropriate anti-infective therapy. A blood culture from a patient with community-acquired pneumonia was found to be positive for *F. tularensis* subsp. *holarctica* biovar II after 10 days of cultivation. Meanwhile, the patient had been treated with ceftriaxone, followed by sultamicillin and clindamycin. The patient continued suffering from fever of up to 40.7 °C and rising C-reactive protein (CRP) for 4 days before the fever and CRP declined. The isolated strain was later tested and found to be resistant to the antibiotics used. The present case underlines that *F. tularensis* subsp. *holarctica* infections may cause severe symptoms but mostly have a favourable outcome.

Introduction

Tularaemia is a re-emerging and potentially severe zoonosis occurring in the northern hemisphere. It is caused by *Francisella tularensis*, an extremely infectious small Gram-negative rod. The true reservoir of *F. tularensis* is unknown, but transmission to humans is mostly related to lagomorphs, rodents, arthropods or water. Only two subspecies of *F. tularensis* cause the vast majority of clinical tularaemia in mammals: *F. tularensis* subsp. *tularensis* (type A), which is endemic in North America, and *F. tularensis* subsp. *holarctica* (type B), which is found in many countries of the holarctic region. Both subspecies show different mortality and virulence patterns. Type B strains can be further divided into biovar I (erythromycin sensitive), biovar II (erythromycin resistant) and biovar japonica. Erythromycin-resistant strains have so far been reported from eastern Europe, western Siberia and Scandinavia.

Case report

In September 2008, a 37-year-old male presented with acute onset of fever (40.7 °C), shivering, a dry cough and weakness. He worked as a landscaper in a rural region in the eastern German state of Saxony. He had removed wood from little forest creeks with bare hands and mowed a lawn in the forest using a petrol grass trimmer.

Radiography revealed an infiltration in the right lung (Fig. 1). Laboratory findings included an elevated white

blood cell count (12 000 cells μl^{-1} , normal range 4000–11 000 cells μl^{-1}) and elevated C-reactive protein (CRP) (40 mg l^{-1} , normal <5 mg l^{-1}). Cultures of blood, urine and tracheal aspirate were negative after 3 days of cultivation.

Community-acquired pneumonia was diagnosed and treatment with 2 g ceftriaxone intravenously once daily was started. During the next 4 days the patient continued suffering from fever exceeding 39 °C and his CRP level rose to 115 mg l^{-1} . Despite the high fever, the patient did well and had no haemodynamic problems.

Due to severe dental caries, an additional focus in the mouth was assumed and the patient received a combination therapy consisting of 750 mg sultamicillin orally twice daily and 600 mg clindamycin orally three times daily for 7 days, while ceftriaxone therapy was stopped. During the next few days his temperature normalized and his CRP level declined. A stomatological examination showed eight destroyed and not conservable teeth and two inflamed tooth roots, all were resected. But there was no abscess or other putrid infection accounting for the septic temperatures.

On day five of cultivation, the initially negative blood culture showed bacterial growth, on day ten *F. tularensis* was identified. Subspecies *F. tularensis* subsp. *holarctica* was identified by PCR targeting the RD (region of difference) gene locus 1 and by 23S rRNA gene sequencing (Broekhuijsen *et al.*, 2003; Splettstoesser *et al.*, 2010). The isolated strain shared 100 % homology with the common laboratory reference strain *F. tularensis* subsp. *holarctica* live vaccine strain (2172 bp analysed). On day 11 a

Abbreviation: CRP, C-reactive protein.

Fig. 1. Radiography of the chest showing an infiltration in the right lung.

commercially available ELISA test (Virion/Serion) detected elevated *F. tularensis* IgM and IgG antibodies (37 U ml^{-1} and 76 U ml^{-1} , respectively, normal $<10 \text{ U ml}^{-1}$).

As was to be expected, the isolated strain was resistant to ampicillin/sulbactam (sultamicillin) ($\text{MIC} >256 \text{ mg l}^{-1}$) and clindamycin ($\text{MIC} >256 \text{ mg l}^{-1}$), but sensitive to ciprofloxacin ($\text{MIC} 0.012 \text{ mg l}^{-1}$), doxycycline ($\text{MIC} 0.38 \text{ mg l}^{-1}$) and gentamicin ($\text{MIC} 0.125 \text{ mg l}^{-1}$) when tested by Etest (with the method described by Tomaso *et al.*, 2005). The isolate was completely resistant to erythromycin: $\text{MIC} >256 \text{ mg l}^{-1}$ (Fig. 2). The MIC for ceftriaxone was $>32 \text{ mg l}^{-1}$.

On day 11 the patient had recovered completely and was released from the hospital. He was prescribed ciprofloxacin for another 10 days for eradication of the tularaemia infection. Radiography after 3 weeks showed a diminished infiltration and the patient developed no relapse symptoms during the following year.

Discussion

From 1975 to 2004, there was only one reported tularaemia case in the German state of Saxony and an annual mean of three reported cases in Germany, with an increase since 2005 (Splettstoesser & Kopf, 2008). Hence, *F. tularensis* initially had not been considered as the causative agent in our patient. Due to the slow growth of the bacteria in culture medium, the correct diagnosis was not established before day 10 of the treatment and the patient had been treated only with inappropriate antibiotics. The course of the patient's disease illustrates the remission of septic pneumonic tularaemia caused by *F. tularensis* subsp. *holarctica* biovar II without appropriate antibiotic treatment. Clindamycin and sultamicillin are generally not

Fig. 2. Erythromycin resistance of the isolated *F. tularensis* subsp. *holarctica* strain, determined by Etest.

effective in tularaemia. According to the European Committee on Antimicrobial Susceptibility Testing an $\text{MIC} >2 \text{ mg l}^{-1}$ for ceftriaxone means resistance, if breakpoints for *Enterobacteriaceae* and non-species related breakpoints are considered, but there are no special standards for *Francisella* (http://www.eucast.org/clinical_breakpoints). Intravenous doses of 2 g ceftriaxone can produce peak serum concentrations of $258 \pm 40 \text{ mg l}^{-1}$ and trough concentrations after 24 h of $12 \pm 4 \text{ mg l}^{-1}$ (Borner *et al.*, 1985). It might be postulated that some *in vivo* activity of the antibiotics cannot be ruled out, especially for ceftriaxone. In systematic *in vitro* studies, ceftriaxone was found both to be effective (Baker *et al.*, 1985) or ineffective (Tomaso *et al.*, 2005) against *F. tularensis*, but it has been reported to fail in clinical manifestations of tularaemia (Cross & Jacobs, 1993). Remission started after 4 days of ceftriaxone; this may be compatible with an at least partial antibiotic activity. In a retrospective study of 234 cases from Sweden the fever lasted for a mean of 3.2 days after the initiation of antibiotic therapy (Eliasson & Bäck, 2007). However, a remission – spontaneous without any or with inappropriate antibiotic treatment – of an infection and even pneumonia with the type B subspecies does not seem unlikely in accordance to the literature (Siret *et al.*, 2006).

Pneumonia caused by *F. tularensis* subsp. *tularensis*, which is almost without exception found in America, had a fatality rate of approximately 30 % during the pre-antibiotic era. *F. tularensis* subsp. *holarctica*, which occurs throughout the northern hemisphere, seems to be less virulent (Eliasson & Bäck, 2007) – as proved in our case.

Given the low number of reported cases, there is a remarkable seroprevalence in Germany and other countries.

Investigations in Germany and Spain revealed 0.23 % and 0.19 % positive sera, respectively, in representative adult population groups (Porsch-Özcürümez *et al.*, 2004; Gutiérrez *et al.*, 2003). A recent study from the southern German urban population of Leutkirch combining a sensitive screening assay with a highly specific confirmative immunoblot test showed a tenfold higher percentage: 2.3 % of the 2416 people screened tested positive for antibodies against *F. tularensis* (Splettstoesser *et al.*, 2009).

This means that the majority of tularemia infections may not be detected: there may be unapparent or slight courses, as well as severe and symptomatic infections that are treated with antibiotics without being identified as tularemia.

Pneumonic tularemia is mainly acquired by inhalation of aerosols containing viable bacteria. The mowing of lawns, brush cutting, hunting and working as a farmer have been identified as risk factors (Feldman *et al.*, 2003; Jenzora *et al.*, 2008; Hauri *et al.*, 2010). Infected animal carcasses or excrements in the lawn are considered the source of infective aerosols during mowing. The subspecies *F. tularensis* subsp. *holarctica* seems to have an additional reservoir in water. Our patient may have inhaled the bacteria by mowing or during working in or next to the small creeks he had to clean.

The therapy of choice includes aminoglycosides, tetracyclines and ciprofloxacin. Whereas some authors described successful treatment with erythromycin (Hoel *et al.*, 1991; Harrell & Simmons, 1990), this substance would be ineffective in persons infected with *F. tularensis* subsp. *holarctica* biovar II. For this reason, most recommendations for empiric anti-infective therapy in community-acquired pneumonia would not include pneumonic tularemia caused by this biovar. Currently, about 40 % of all strains isolated in Germany belong to this category (W. D. Splettstoesser & E. Seibold, unpublished data).

In conclusion, rare disorders such as tularemia are often not considered as a differential diagnosis in patients with community-acquired pneumonia. A detailed history, including occupational or other outdoor activities, especially in the forest, and contact with hares, rabbits or other animals, may be crucial, and adequate information should always be sent to the laboratory. This information may help prevent aerogenic infection of the laboratory staff and it may be one of the crucial steps on the way to the correct diagnosis, because the detection and identification of this fastidious bacterium by classical or routine bacteriological methods is cumbersome, difficult and often unsuccessful. Thus, serology is still a cornerstone in the confirmation of tularemia.

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