

ORIGINAL ARTICLE

Rickettsia felis infection in Sweden: Report of two cases with subacute meningitis and review of the literature

A. LINDBLOM^{1,2}, KRISTOFFER SEVERINSON³ & KENNETH NILSSON^{1,3,4,5}

From the ¹Unit of Infectious Diseases, Department of Medical Sciences, Uppsala University, Uppsala, ²Department of Infectious Diseases, Falu County Hospital, Falun, ³Unit of Clinical Microbiology, Department of Medical Sciences, Uppsala University, Uppsala, ⁴Department of Clinical Microbiology, Falu County Hospital, Falun, and ⁵Center of Clinical Research Dalarna, Falun, Sweden

Abstract

Two patients with headache and pleocytosis were diagnosed with *Rickettsia felis* infection using a polymerase chain reaction of cerebrospinal fluid and serological testing. Sequencing of the amplified products showed that they were 99–100% homologous to *R. felis*. These cases add to our knowledge of the clinical manifestations, as well as the geographical distribution, of this spotted fever agent.

Introduction

Rickettsioses are arthropod-borne diseases caused by obligate intracellular Gram-negative bacteria. The genus *Rickettsia* is traditionally classified into 2 groups, the spotted fever group (SFG) and the typhus group [1]. SFG members are transmitted mainly by ticks, but also by fleas and mites, and in Europe, 8 species have been described as emerging pathogens [2].

Rickettsia helvetica, associated with *Ixodes ricinus* ticks, is the only *Rickettsia* isolated in Sweden [3]. A handful of reported patients infected by this agent have presented with symptoms of fever and sometimes a rash and/or an eschar, but both a more severe clinical picture and meningitis have recently been demonstrated [4,5].

In 1990, *Rickettsia felis* was detected in cat fleas (*Ctenocephalides felis*), which serve as the main reservoir and vector and play a role in the transmission of human illness [6]. Since then, human cases have been confirmed using polymerase chain reaction (PCR) and/or serological tests, in patients from Texas (1994), South/Central America, Europe, Africa and Asia [1–2,7]. The illness is typically mild and characterized by fever, headache, myalgia and cutaneous manifestations (rash, eschar, ulcer), but lymphadenopathy and neurological manifestations

(photophobia, hearing loss), abdominal symptoms (nausea, diarrhoea) and pneumonia may also occur [1,7,8].

As a consequence of the previously reported isolation of *R. helvetica* from a patient with meningitis [5], we have an ongoing project searching for fastidious organisms in patients with a similar clinical picture. As a result of this study we now report the cases of 2 patients with subacute meningitis where the clinical investigation indicated a viral cause, but PCR later confirmed the presence of another spotted fever *Rickettsia*, *Rickettsia felis*, in the cerebrospinal fluid (CSF). *R. felis* has not previously been reported in Sweden in any vector or as a cause of infection.

Case reports and methods

Patient 1, a 47-y-old previously healthy man, was hospitalized in May 2008 at Falu County Hospital (Falun, Sweden). A week earlier he had begun to feel ill, reporting headache and tiredness. The headache became progressively worse, especially at night. He had no other signs of infection except a slight sore throat. He felt feverish the first days of illness, but did not check his body temperature. He started

Correspondence: K. Nilsson, Unit of Clinical Microbiology, Department of Medical Sciences, Uppsala University Hospital, SE-751 85 Uppsala, Sweden. Tel.: +46 18 611 00 00. Fax: +46 18 50 81 27. E-mail: kenneth.nilsson@medsci.uu.se

(Received 25 June 2010; accepted 29 June 2010)

vomiting and felt sensitive to light and sound the day he sought care. He knew he had been bitten by a tick 8 months earlier, but had not observed any later tick bite. He had not had any contact with dogs or cats. Examination showed a man who was pale and weak with a severe headache. He had no fever, but a slight redness in the throat. Laboratory tests revealed slightly elevated C-reactive protein (23 mg/l) and white blood cell (WBC) count ($10.9 \times 10^9/l$), and normal haemoglobin (136 g/l) and platelet cell count ($250 \times 10^9/l$) values. The CSF showed a pleocytosis of 32×10^6 mononuclear cells/l and albumin was elevated (407 mg/l), but the ratio of albumin between serum and CSF was normal. Computed tomography with contrast fluid was normal. Cultures from blood and CSF, and tests for herpes and enteroviruses, tick-borne-encephalitis (TBE) virus and varicella were all negative. Tests for antibodies in serum and CSF against *Borrelia burgdorferi* were negative. He was diagnosed as having serous meningitis and was treated with pain killers for the headache and received fluid intravenously (i.v.). After 2 days in hospital, he felt better and returned home. He received no antibiotics. One year later he was still healthy.

Since no agent had been detected, he was retrospectively included in the study, after giving his informed consent; PCR of his previously frozen CSF demonstrated the presence of rickettsial DNA.

Patient 2, an 89-year-old woman, was hospitalized in May 2008 at Uppsala University Hospital (Uppsala, Sweden) for fever and back pain. She lived near the sea in her own apartment and took care of the household herself. Her history included asthma and a newly discovered atrial fibrillation. On admission she was tired, a bit confused, but without focal neurology. Her temperature was 38.5°C, blood pressure 149/80 mmHg and she had an irregular pulse at 130/min. There was no lymphadenopathy or cutaneous lesion. She had pains over the left kidney lobe. Her WBC count was $9.8\text{--}12.6 \times 10^9/l$, haemoglobin 123 g/l, platelet cell count $204\text{--}419 \times 10^9/l$, and her C-reactive protein level was 139 mg/l. After blood and urine cultures were performed, treatment was initiated using piperacillin/tazobactam and gentamicin i.v. Urine culture showed >100,000 bacteria/ml of *Klebsiella pneumoniae* as a result of a urinary tract infection. However, the patient remained weak with progressive mental confusion. Computed tomography of the brain was normal. CSF showed 16×10^9 mononuclear cells/l, normal albumin, slightly elevated lactate (3.0 mmol/l) and lowered glucose ratio (<0.5). No other source of infection or abscesses was found. On suspicion of meningitis, the antibiotic treatment was changed after 2 days to cefotaxime and ampicillin i.v. in doses for meningitis, after which she slowly recovered; she left the hospital after

10 days of treatment. However, cultures from CSF and tests for herpes and enteroviruses were negative. As for patient 1, following informed consent, she was retrospectively included in the study.

Samples of CSF from the 2 patients, previously stored at -20°C in a regular freezer, were thawed. Bacterial DNA was extracted using the NucliSens easyMAG automated extraction platform (bio-Mérieux, Durham, NC, USA) according to the manufacturer's instructions. Rickettsial DNA was amplified using a genus-specific quantitative real-time PCR assay with the probe (Taqman) and primers targeting the *gltA* gene, as previously described [9]. The PCR reactions were performed in duplicate in a Rotor-Gene 3000 (Corbett Research, Sydney, Australia) and in each reaction, 0.25 µl LC Uracil-DNA glycosylase (Roche) was included to reduce the risk of contamination. As a standard, the 74-base pair (bp) fragment of *R. helvetica*, amplified in the real-time PCR reaction, was ligated into a pCR 4-TOPO vector and transformed into One Shot TOP10 chemically competent *Escherichia coli* following the manufacturer's instructions (TOPO TA Cloning® Kit for Sequencing, Invitrogen). Ten-fold serial dilutions of extracted plasmids were used to establish standard curves for the PCR runs. The quantification was linear over a range of 10 to 10^9 copies, and the detection limit was shown to be 1–10 copies per reaction.

The positive patient samples were further analysed using 2 nested PCR assays that amplify the 17-kDa and *ompB* gene fragments, as previously described [10,11]. Purified DNA of *R. helvetica* was used in the nested assays as the positive control [3]. Amplification was carried out in a DNA thermal cycler (GeneAmp PCR System 9700, PE Applied Biosystems), and expected fragment sizes were confirmed using gel electrophoresis (2% agarose). Direct cycle sequencing analysis of both strands was performed using an automatic Hitachi 3100 Avant Plus Genetic Analyzer (Applied Biosystems, Tokyo, Japan) and at the Centre for Genomics and Bioinformatics, KI, Stockholm (KI Seq).

For microimmunofluorescence (MIF), bacterial antigen in the form of *R. helvetica*-infected Vero cells supplemented with 10% yolk sac solution was applied to each well of the microscope slides, dried, fixed in acetone and incubated with serial dilutions of serum or CSF, as previously described [12]. As positive control, a positive serum sample from a patient with proven infection with *Rickettsia conorii* with end-point IgG titres of 1:160, provided by the Swedish Institute for Infectious Disease Control, and serum from another patient with proven end-point IgG titres of 1:80 to *R. helvetica* were used. IgG antibodies were detected using fluorescein isothiocyanate-conjugated (FITC) γ -chain-specific

polyclonal rabbit anti-human IgG (Ref.: F0202; Dako, Denmark).

A lateral flow coal immunochromatographic assay (LFCIA) was used to confirm the presence of IgG antibodies reactive with spotted fever Rickettsia (SFR). *R. helvetica* cultured in Vero cells and purified by Percoll centrifugation were used as diagnostic antigen and applied as small droplets on a nitrocellulose strip; 100 µl serum from patient 1 in a titre 1/100 was added on a pad at one end of the strip. After loading, 100 µl Tris 10 mM coal-conjugated γ -chain-specific polyclonal rabbit anti-human IgG was added, and the result was read after a few minutes. If the spot turned black, it indicated serum-antibodies reactive with rickettsial antigen. Vero cells only were used as the negative control [13].

Results

For both patients, CSF samples that were positive in the real-time PCR with a Taqman probe produced amplicons in nested PCR of partial regions of the 17-kDa and *ompB* genes. The sequences obtained were 151 bp (17-kDa) and 265 bp (*ompB*) (Figure 1). Sequence analysis of these amplicons revealed the presence of *R. felis*. For patient 2, it was not possible to amplify the 17-kDa gene, probably because the amount of DNA was near the detection limit and further because we had no more extracted sample. The sequences obtained shared 99–100%

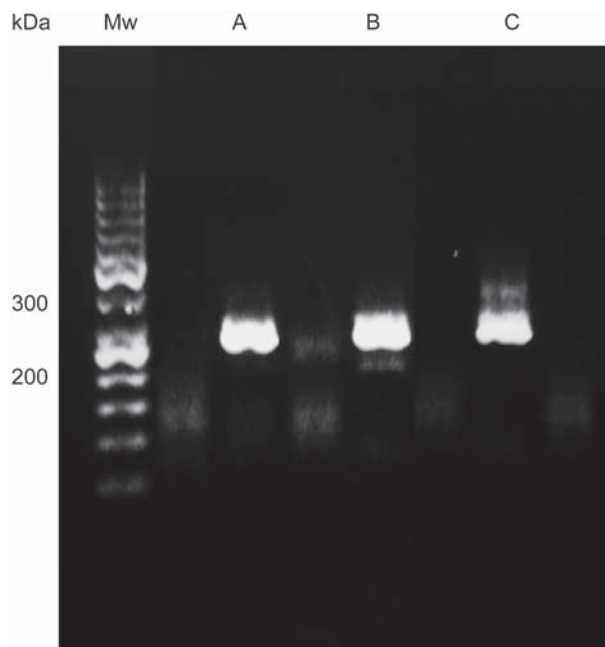


Figure 1. Ethidium bromide stained agarose gel (2%). Lane Mw: molecular weight marker; lanes A and B: *ompB*, show PCR amplicons (265 bp) from patients 1 and 2; lane C: positive control (*R. helvetica*).

similarity with the corresponding gene sequences of *R. felis* (GenBank accession numbers **DQ102709.1** (17-kDa) and **AF210695.1** (*ompB*)) and showed significant nucleotide differences from the other rickettsiae in the spotted fever group. The sequences obtained from patient 1 (17-kDa, *ompB*) and patient 2 (*ompB*) have been deposited at GenBank; accession numbers **GQ182891** and **GQ182892**.

MIF showed IgG antibody reactivity with *R. helvetica* as antigen and representative of the spotted fever group, with end titres of 1:64 in the early phase serum sample after 8 and 6 days for patients 1 and 2, respectively. All negative controls were negative. No other sera were available. LFCIA, only tested in patient 1, confirmed the presence of specific IgG antibodies reactive with spotted fever Rickettsia (Figure 2).

Discussion

Both patients presented symptoms of fever, headache and tiredness, but no manifestations in the skin or lymph nodes were seen. Their headaches were pronounced, and central nervous system involvement was confirmed by lumbar puncture showing pleocytosis. The PCR analysis of CSF verified the presence of *R. felis*. According to the standard curve, the Ct (cycle threshold) values indicated only 1–10 DNA copies/µl in the samples. However, amplicon contamination seems very unlikely, as we have never amplified, cultured or used *R. felis* as an antigen in serological assays in our laboratory. The obtained 17-kDa and *ompB* sequences have the same length as the amplified products from *R. helvetica*, but differ in 13 and 11 bp, respectively, from *R. helvetica*, which indicates another species. Besides, all negative controls were negative, and DNA amplification was performed using primers targeting 3 different genes. The serological testing also confirmed the presence of anti-rickettsial IgG antibodies, but because only early phase sera were available, it was not possible to investigate seroconversion.

The previously reported patients from Yucatan, Mexico, presented neurological symptoms such as photophobia and hearing loss, but in those cases *R.*

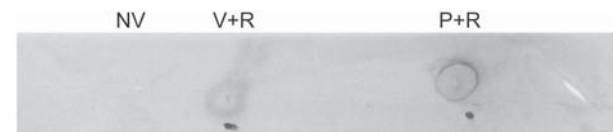


Figure 2. Lateral flow coal immunochromatographic assay (LFCIA) strip for detecting anti-spotted fever rickettsial antibodies. NV: negative Vero cells control; the 2 dark spots represent Vero cell-cultured rickettsia (V+R) and Percoll-purified *R. helvetica* (PR) bound to an anti-rickettsial IgG and coal-conjugated anti-human IgG complex.

felis was not detected in the CSF [8]. They also had abdominal symptoms and cutaneous manifestations, which our patients 1 and 2 did not show. Patient 1 was not treated with antibiotics, but still recovered and the symptoms resolved within 2 weeks. Other reports support the notion that the symptoms may be mild to moderate and non-specific and that this is sometimes a self-healing disease; for these reasons it is easily confused with other febrile illnesses [8,14]. The clinical picture of patient 2 was more complex and also influenced by a concomitant urinary tract infection.

R. felis, which has not previously been reported in Scandinavia, is reported to have a worldwide distribution and is maintained in nature by transstadial and trans-ovarian transmission of the cat flea (*C. felis*) [1,2]. Fewer than 70 patients have been reported ill with *R. felis* worldwide [7]. The number of reported findings in the vector is significantly higher, and it has also been reported from many countries in Central Europe [8,15].

The present report confirms that *R. felis* may cause infection of the central nervous system and may occur in Sweden, where previously only *R. helvetica* has been reported; it has also recently been shown to cause meningitis [5]. These findings underline the importance of further studies on the prevalence and incidence of human rickettsial infections in Sweden, together with studies investigating the diversity of rickettsioses identified in the ecosystem.

Acknowledgements

This study was supported by grants from the Uppsala-Örebro-Regional Research Council (25021); Centre of Clinical Research Dalarna (9028) and Signhild Engkvists Foundation and Olle Engkvist Byggmästare Foundation (11877).

Declaration of interest: No conflict of interest to declare.

References

- [1] Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev* 2005;18:719–56.
- [2] Broqui P, Parola P, Fournier PE, Raoult D. Spotted fever rickettsioses in southern and Eastern Europe. *FEMS Immunol Med Microbiol* 2007;49:2–12.
- [3] Nilsson K, Lindquist O, Liu AJ, Jaenson TG, Friman G, Pålsson C. *Rickettsia helvetica* in *Ixodes ricinus* ticks in Sweden. *J Clin Microbiol* 2002;37:400–3.
- [4] Nilsson K. Septicaemia with *Rickettsia helvetica* in a patient with acute febrile illness, rash and myasthenia. *J Infect* 2009; 58:79–82.
- [5] Nilsson K, Elfving K, Pålsson C. *Rickettsia helvetica* in patient with meningitis, 2006. *Emerg Infect Dis* 2010;16: 490–2.
- [6] Adams JR, Schmidtman ET, Azad AF. Infection of colonized cat fleas, *Ctenocephalides felis* (Bouché) with a *Rickettsia*-like microorganism. *Am J Trop Med Hyg* 1990; 43:400–9.
- [7] Pérez-Osorio CE, Zavala-Velázquez JE, Arias León JJ, Zavala-Castro JE. *Rickettsia felis* as emergent global threat for humans. *Emerg Infect Dis* 2008;14:1019–23.
- [8] Zavala-Castro J, Zavala-Velázquez J, Walker D, Pérez-Osario J, Peniche-Lara G. Severe human infection with *Rickettsia felis* associated with hepatitis in Yucatan, Mexico. *Int J Med Microbiol* 2009;299:529–33.
- [9] Stenos J, Graves SR, Unsworth NB. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group *Rickettsia*e. *Am J Trop Med* 2005;73: 1083–5.
- [10] Choi YJ, Lee SH, Park KH, Koh YS, Lee KH, Balk HS, et al. Evaluation of PCR-based assay for diagnosis of spotted fever group rickettsiosis in human serum samples. *Clin Diagn Lab Immunol* 2005;12:59–63.
- [11] Leitner M, Yitzhaki S, Rzotkiewicz S, Keysari A. Polymerase chain reaction-based diagnosis of Mediterranean spotted fever in serum and tissue samples. *Am J Trop Med* 2002; 67:166–9.
- [12] Elfving K, Lindblom A, Nilsson K. Seroprevalence of *Rickettsia* spp. infection among tick-bitten patients and blood donors in Sweden. *Scand J Infect Dis* 2008;40:74–7.
- [13] Tamura A, Urakami H, Tsuruhara T. Purification of *Rickettsia tsutsugamushi* by Percoll density gradient centrifugation. *Microbiol Immunol* 1982;26:321–8.
- [14] Velázquez-Zavala JE, Ruiz-Sosa JA, Sanchez-Elias RA, Becerra-Cermonia G, Walker DH. *Rickettsia felis* rickettsiosis in Yucatán. *Lancet* 2000;23:1079–80.
- [15] Renvoisé A, Joliot AY, Raoult D. *Rickettsia felis* infection in man, France. *Emerg Infect Dis* 2009;15:1126–7.