doi:10.1111/ene.12218

Bell's palsy and sudden deafness associated with *Rickettsia* spp. infection in Sweden. A retrospective and prospective serological survey including PCR findings

K. Nilsson[a,b,c], K. Wallménius[a], S. Hartwig[d], T. Norlander[d] and C. Påhlson[a]

[a]*Department of Medical Sciences, Section of Clinical Microbiology, Uppsala University, Uppsala;* [b]*Department of Medical Sciences, Section of Infectious Diseases, Uppsala University, Uppsala;* [c]*Centre of Clinical Research, Falu Hospital, Falun; and* [d]*Department of Otorhinolaryngology, Falu Hospital, Falun, Sweden*

Keywords:
Bell's palsy, deafness, neuritis, PCR, serology, spotted fever rickettsia

Received 19 January 2013
Accepted 16 May 2013

Background and purpose: Sixty patients with facial palsy and 67 with sudden deafness were retrospectively or prospectively examined for serological evidence of rickettsial infection; in six cases where cerebrospinal fluid was available, patients were also examined for presence of rickettsial DNA.

Methods: Rickettsial antibodies were detected in single or paired serum samples using immunofluorescence with *Rickettsia helvetica* as the antigen and in four cases also using western blot. Using PCR and subsequent direct cycle sequencing, the nucleotide sequences of the amplicons (17 kDa protein gene) in cerebrospinal fluid were analysed.

Results: Five out of 60 (8.3%) patients with facial palsy and eight of 67 (11.9%) with hearing loss showed confirmative serological evidence of infection with *Rickettsia* spp. An additional three and four patients in the facial palsy and hearing loss groups, respectively, showed evidence of having a recent or current infection or serological findings suggestive of infection. In four cases, the specificity of the reaction was confirmed by western blot. An additional 70 patients were seroreactive with IgG or IgM antibodies higher than or equal to the cut-off of 1:64, whereas 37 patients were seronegative. Only two of 127 patients had detectable antibodies to *Borrelia* spp. In three of six patients, rickettsial DNA was detected in the cerebrospinal fluid, where the obtained sequences (17 kDa) shared 100% similarity with the corresponding gene sequence of *Rickettsia felis*.

Conclusions: These results highlight the importance of considering *Rickettsia* spp. as a cause of neuritis, and perhaps as a primary cause of neuritis unrelated to neuroborreliosis.

Introduction

The cause of peripheral facial nerve palsy (FNP) or Bell's palsy, as well as that of sudden deafness (SD), is often unknown. In Sweden, between 0.1 and 0.3 per 1000 of the population are diagnosed every year with one of these disorders. It is known that palsies can be associated with reactivated herpes simplex virus or a specific immune response to infection. Other possible explanations are varicella zoster virus or Lyme borreliosis (LB), especially in children or in bilateral palsy, or sometimes sarcoidosis. Sudden deafness, on the other hand, is thought to be due to a viral and/or bacterial infection, trauma, neoplasms, otologic disease, or vascular or haematological disorders. The majority of the cases remain idiopathic [1,2].

Rickettsioses have not been implicated as causative agents but, based on serological evidence, have occasionally been associated with these diseases [3,4]. Rickettsioses are systemic diseases with symptoms caused by vasculitis, which results from proliferation of rickettsiae in vascular endothelial cells. Of the spotted fever rickettsiae (SFR), neurological manifestations occur in some cases; meningitis or involvement of the peripheral nervous system has typically been reported in cases presenting severe systemic manifestations [4–6]. *Rickettsia felis*, an SFR, is reported to have worldwide distribution, with cat fleas (*Ctenophalides felis*)

Correspondence: K. Nilsson, Department of Medical Sciences, Clinical Microbiology, Uppsala University, SE-751 85 Uppsala, Sweden (tel.: +46 18 611 00 00; fax: +46 18 50 81 27; e-mail: kenneth.nilsson@medsci.uu.se).

EUROPEAN JOURNAL OF NEUROLOGY
THE OFFICIAL JOURNAL OF THE EUROPEAN FEDERATION OF NEUROLOGICAL SOCIETIES

being the main reservoir and vector in disease transmission to humans. Its pathogenic role in humans has been demonstrated through PCR and serology in about 70 cases, and the typical symptoms are characterized by fever, headache, myalgia, cutaneous manifestations (rash, eschar), lymphadenopathy and neurological manifestations (photophobia, hearing loss) [7–9]. *Rickettsia helvetica* is the only reported tick-transmitted SFR in Sweden (besides a single reported finding of *Rickettsia sibirica)*, occurring in approximately 9%–10% of *Ixodes ricinus* ticks [10]. A handful of infected patients have presented with a febrile illness similar to *R. felis*, occasionally myocarditis but in two cases meningitis, where the organism was also isolated from cerebrospinal fluid (CSF), including one patient with concomitant reactivation of herpes virus 2 [11–13]. In an ongoing investigation of patients with different neurological manifestations, two cases with subacute meningitis caused by *R. felis* were also found recently [14]. However, thus far *R. felis* has not been reported in any vector in Sweden.

Here one retrospective and one prospective study of a total of 127 patients diagnosed at the Otorhinolaryngology Clinic, Falun Hospital, Sweden, and Uppsala University Hospital, Uppsala, Sweden, and presenting symptoms associated with the seventh and eighth cranial nerves are reported as well as serological and molecular evidence of *Rickettsia* spp. infection.

Material and methods

Patients, serum and cerebrospinal fluid

Retrospective study (Study 1)
Samples of serum from 40 patients diagnosed with FNP and 30 patients presenting with SD, previously stored at −20°C in a regular freezer, were thawed and re-examined for the presence of rickettsial antibodies. Five of the patients with FNP and one with SD had undergone lumbar puncture and were examined for *Rickettsia* spp. using PCR. Cerebrospinal fluid samples were taken at the same time as the serum samples. The samples had been collected from 2009 to 2011 and diagnoses had previously been made at the Otorhinolaryngology Clinic, Falun Hospital, and in some cases at Uppsala University Hospital. The patients were between 6 and 84 years of age (34 female and 36 male). Most patients had sought medical care within 1 week after symptom onset, with a range up to 3 months, and were sampled for serum at the time of the first doctor visit. In cases where one or more convalescent serum samples had been collected, they were examined in the same manner. The vast majority of patients had been treated with prednisolone or local treatment (drops, ointment, taping or humidity chamber), whilst a smaller number had received treatment with antiviral or antibacterial drugs.

Prospective study (Study 2)
A total of 57 patients, of whom 20 showed FNP and 37 had sudden hearing loss, at the Otorhinolaryngology Clinic, Falun Hospital, were sampled for two sera (S1 and S2): sample 1 (S1) on enrolment day at the time of the first doctor visit and sample 2 (S2) collected 6–8 and up to 24 weeks later. All patients with FNP had severe dysfunction corresponding to grade V or VI according to the House−Brackman facial nerve grading system. SD was defined as sensorineural hearing loss over three contiguous pure-tone frequencies of 30 dB or more with a duration of less than 72 h. All sera were examined for the presence of rickettsial antibodies, in the same manner as in Study 1. The age distribution was between 23 and 74 years (27 female and 29 male patients). The distribution of symptom durations and applied treatments was similar to that in Study 1. PCR on the CSF of these patients was not performed because it is not usually part of the normal investigation and ethical permission had not been authorized for expanded diagnostics. In both Study 1 and Study 2, data on tick bite, symptoms, laboratory findings and initial treatment were obtained from the medical records (after informed consent) based on the initial examination and subsequent follow-up. Prior to or concurrent with our study, sera were analysed for antibodies against *Borrelia* spp.; in Study 2, paired sera were used.

Statistical analysis

For continuous variables, standard parametric statistics (confidence interval according to Fleiss with Yates's correction) giving the mean ± 95% confidence interval (CI) were used. Statistical analyses were conducted using Predictive Analytics Software (PASW) Statistics version 20 (IBM, Portsmouth, NH, USA).

DNA extraction

Bacterial DNA was extracted from CSF using the NucliSens easyMAG automated extraction platform (bioMérieux, Durham, NC, USA), according to the manufacturer's instructions.

PCR

The spotted fever group of rickettsiae was assayed using genus-specific quantitative real-time PCR with probe and primers targeting the *glt*A gene, as previ-

ously described [15]. A pCR4-TOPO plasmid containing the cloned 74 bp fragments of the *glt*A gene was used in 10-fold serial dilutions as a standard for the PCR run. The real-time PCR assay was performed in a Rotor-Gene 3000 (Corbett Research, Sydney, NSW, Australia) using LC Taqman Master kit (Roche Diagnostics, GmbH Mannheim, Germany). Reagent controls containing no DNA were not amplified. The positive samples were further analysed using PCR assays that amplify the 17 kDa, *omp*B, *omp*A and *glt*A genes, as previously described [16–20]. Conventional and nested PCR were performed in a DNA thermal cycler [GeneAmp PCR System 9700 (PE Applied BioSystems, Carlsbad, CA, USA)], and expected fragment sizes were confirmed using gel electrophoresis (2% agarose, 1% ethidiumbromide). Confirmation of fragment size was based on a standard DNA molecular weight marker (Invitrogen, Carlsbad, CA, USA). As a negative control, sterile water was included in each amplification trial. As a positive control, purified DNA of *Rickettsia conorii* was used (AmpliRun Rickettsia Conorii DNA Control, Vircell) as well as extracted DNA from *R. helvetica* isolated from an *I. ricinus* tick [21].

Direct cycle sequencing analysis of both strands of amplicons was performed using an automatic Hitachi 3100 Avant Plus Genetic Analyzer (Applied Biosystems, Tokyo, Japan). For species identification, pairwise similarities to and differences from other rickettsiae in the spotted fever group were examined using BLAST analysis. Multiple sequence alignments were conducted using BioEdit version 7.0.9 and ClustalW.

Serology

For microimmunofluorescence, *R. helvetica*-infected Vero cells supplemented with 10% yolk sac solution were applied as the bacterial antigen to each well of the microscope slides, and then dried, fixed in acetone and incubated with serial dilutions of serum, as previously described [22]. As positive controls, a serum sample from a patient with a proven end-point IgG/IgM of 1:512/1:128 to *R. helvetica* was used. A human blood donor serum was used as a negative control. IgG and IgM antibodies were detected by fluorescein-isothiocyanate-conjugated γ- and μ-chain-specific polyclonal rabbit anti-human IgG and IgM (ref. F0202 and F0203, Dako, Glostrup, Denmark). Before examination of IgM, the serum was pretreated with rheumatoid factor absorbent (Immunkemi, Stockholm, Sweden). Titres < 1:64 were considered negative for IgG/IgM. As per Centers for Disease Control and Prevention (Atlanta, GA, USA), a confirmed case was defined as a fourfold or greater rise in IgG titre between acute phase (S1) and convalescent phase (S2) sera taken at the onset and 4–6 weeks later and tested in parallel. Single IgG end-point titres of ≥1:256 were considered presumptive evidence of recent or current infection and defined as a probable case. Single IgG and/or IgM end-point titres ≥1:64 and <1:256 were regarded as supportive evidence indicative of either past infection or early response to infection whilst persisting positive IgG titres with or without IgM reactivity were considered as past infection. Persisting IgM antibodies alone were interpreted as non-specific cross-reactivity due to exposure to other organisms or autoimmune responses or possibly a sign of a previous exposure. Laboratory evidence of a clinical or subclinical infection with *Borrelia burgdorferi* was based on comparison of acute and, in cases where applicable, convalescent sera, using a commercial enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions [Euroimmun AG (Aktiengesellschaft), Lübeck, Germany], or the Lyme Borreliosis ELISA kit (Oxoid Ltd, Basingstoke, Hampshire, UK).

Western blot

Sera from four of the IgG-positive patients (P12, P23, P29 and P44 in Table 2) in Study 2 were diluted to 1:500 and tested for western blot (WB) with *R. helvetica* whole-cell antigen as previously described [23]. As the positive control a serum from rabbit immunized with purified *R. helvetica* was used, and the secondary antibody alone served as the negative control.

Absorption study

One of the samples (P23) with a titre ≥512 was selected for absorption. A minor aliquot of the undiluted serum sample was mixed in an equal volume with a concentrated Vero cell derived whole-cell *R. helvetica* antigen suspension, and then diluted in phosphate-buffered saline to a dilution of 1:600, incubated during gentle shaking for 5.5 h at 37°C, and then stored overnight at 5°C and centrifuged for 5 min at 20 000 *g* before serial twofold dilutions were made (1:600, 1:1200 and 1:2400) and used for WB [24]. The end-points of both absorbed and unabsorbed dilutions were compared also in immunofluorescence assay (IFA).

Results

Study 1 (retrospective)

Of the 40 patients with facial palsy and 30 with SD, three (R11, R18 and R21) showed a fourfold rise in

Table 1 Study 1 (retrospective). Results of serology and PCR for the *Rickettsia* spp and *Borrelia* spp. in sera S1–S3

No.	Age/sex	Duration of symptoms (days)	S1		S2		Weeks/ S1–S2	S3		Months/ S1–S3	PCR/ CSF	Borrelia IgG/IgM	Treatment	Tick bite
			IgG	IgM	IgG	IgM		IgG	IgM					
Facial palsy														
R1	70/F	2	<64	64	<64	64	1.5	<64	<64	10	NA	neg	P, AV	ND
R2	53/M	40	64	128	NA	NA		NA	NA		NA	neg	LT	ND
R3	72/F	3	64	64	NA	NA		<64	<64	10	NA	neg	P	N
R4	64/M	14	64	512	NA	NA		NA	NA		NA	neg	LT	ND
R5	70/F	7	<64	64	NA	NA		NA	NA		NA	neg	LT	ND
R6	24/M	7	<64	256	NA	NA		NA	NA		neg	neg	P	N
R7	25/M	7	64	512	NA	NA		NA	NA		NA	neg	P	ND
R8	16/F	9	<64	256	NA	NA		NA	NA		neg	neg	LT	N
R9	23/F	3	64	128	NA	NA		<64	<64	11	NA	neg	LT	ND
R10	12/F	3	64	64	NA	NA		NA	NA		NA	neg	LT	Y
R11	25/M	5	<64	256	128	1024	4	64	128	10	pos	G−/M+	AV	N
R12	51/M	2	<64	128	NA	NA		NA	NA		pos	neg	AV	ND
R13	37/F	5	<64	256	64	64	5	NA	NA		NA	neg	P	N
R14	39/F	25	64	512	NA	NA		NA	NA		NA	neg	P	ND
R15	60/M	12	512	128	NA	NA		NA	NA		NA	neg	DC	Y
R16	47/F	4	64	1024	NA	NA		NA	NA		NA	neg	DC, P	ND
R17	38/M	6	64	128	NA	NA		NA	NA		NA	neg	DC	Y
R18	49/M	2	<64	64	128	<64	10	NA	NA		NA	neg	P	ND
R19	25/F	4	<64	256	NA	NA		NA	NA		NA	neg	LT	N
R20	76/M	3	<64	128	NA	NA		NA	NA		NA	neg	LT	ND
R21	28/F	4	<64	256	128	512	7	64	128	4	NA	neg	LT,P	N
R22	69/M	4	<64	128	NA	NA		NA	NA		NA	neg	LT	ND
R23	14/M	7	64	512	NA	NA		NA	NA		NA	neg	LT	ND
R24	6/M	4	64	128	NA	NA		NA	NA		NA	neg	LT	ND
Sudden deafness														
R25	60/M	90	512	<64	NA	NA		NA	NA		NA	neg	P	ND
R26	67/F	2	<64	64	64	<64	4	NA	NA		NA	neg	P	ND
R27	65/F	53	1024	64	NA	NA		NA	NA		NA	neg	LT	ND
R28	55/F	4	64	<32	NA	NA		NA	NA		NA	neg	P	ND
R29	77/F	3	<64	256	NA	NA		NA	NA		NA	neg	P	ND
R30	72/M	3	<64	128	NA	NA		NA	NA		NA	neg	LT	ND
R31	82/F	5	<64	512	NA	NA		NA	NA		NA	neg	P,AV	ND
R32	76/M	3	<64	64	NA	NA		64	128	4	NA	neg	LT	ND
R33	51/M	7	128	128	NA	NA		NA	NA		NA	neg	LT	ND
R34	59/M	2	<64	128	NA	NA		NA	NA		NA	neg	P	ND
R35	46/F	12	<64	256	NA	NA		NA	NA		NA	neg	P	ND
R36	60/F	2	<64	128	NA	NA		NA	NA		NA	neg	P	ND
R37	27/F	9	<64	128	NA	NA		NA	NA		NA	neg	P	Y
R38	71/F	7	64	128	NA	NA		NA	NA		NA	neg	P	ND
R39	12 M	2	<64	128	NA	NA		NA	NA		pos	neg	P	ND
R40	52/F	10	64	64	NA	NA		NA	NA		NA	neg	P	ND
R41	31/M	1	<64	<64	NA	NA		64	<32	12	NA	neg	P	ND
R42	54/M	4	64	512	NA	NA		NA	NA		NA	neg	P	ND
R43	42/F	4	<64	512	64	1028	4	NA	NA		NA	neg	P,AV	N
R44	65/M	6	<64	256	NA	NA		NA	NA		NA	neg	P	N

CSF, cerebrospinal fluid; NA, not applicable; AV, antiviral; DC, doxycycline; neg, negative; pos, positive; P, penicillin; LT, local treatment; ND, no data; N, no; Y, yes; R1–R44, retrospective patient number 1 to 44.

IgG titre judged as confirmative of infection (Table 1). Correspondingly, three patients (R15, R25, R27) had serological findings with IgG titres ≥256 indicating a recent or current infection and judged as probable cases. One patient with SD (R33) showed a single IgG and IgM titre of 128 suggestive of infection. Sixteen of 40 and 10/30 patients, respectively, were seronegative, and 21/40 and 16/30, respectively, were sero-reactive with titres between ≥64 and <256 as a result of early response, past infection or non-specific reactivity (IgM). In both groups, serological interpretation was complicated as in most cases the convalescent serum was lacking.

For three (R11, R12 and R39) of the six patients analysed using PCR, the CSF sample was positive in real-time PCR and also produced amplicons, in

conventional PCR, of partial regions of the 17 kDa protein gene. The obtained, chosen complete sequences for these three patients were 394, 386 and 394 bp (17 kDa), respectively, and analyses of these amplicons shared 100% similarity with the corresponding gene sequence of *R. felis* (GenBank accession number CP000053.1). Due to the limited availability of extracted DNA no amplicons good enough for sequencing in the assays of the *omp*B and *omp*A genes were obtained. No attempt was made to detect DNA in blood or serum samples.

Only one (R11) of 70 patients was seroreactive (IgM) for *Borrelia* spp. as well. The laboratory results of all patients in Study 1 are summarized in Table 1, and the symptoms and the laboratory results of the three PCR-positive patients are summarized in Table 2.

Table 2 Summary of the laboratory findings for the PCR-positive patients in Study 1

Characteristics	R11	R12	R39
Sex, age in years	F, 25	M, 51	M, 12
Previous diseases	None	None	None
Month	Oct	Feb	Aug
Duration of symptoms/days	5	10	3
Duration of facial palsy/days	3	8	NA
Duration of hearing loss/days	NA	NA	5
Fever (°C)	37.4	35.5	36.8
Headache	Yes	Yes	No
Dizziness	No	No	No
Stiff neck/neck pains	No/No	No/Yes	No
Photophobia	No	No	No
Body-aches, pains	No	Yes	No
Rash	No	No	No
Treatment (days)			
AV	10	NA	NA
DC	NA	10 (0.2 g/day)	10 (0.2 g/day)
P	7	NA	60
C-reactive protein (mg/l)	<5	<8	9
WBC count (g/l)	6.3	5.3	7.7
CSF results			
CSF cells total	<2	3	13
Mono	0	3	13
Albumin (mg/l)	Normal	Normal	Normal
Blood glucose ratio	Normal	Normal	Normal
PCR results			
Real-time PCR			
*glt*A	pos	pos (2)	pos (2)
PCR+seq-id			
*omp*B	neg	ND	ND
*omp*A	ND	ND	neg
17 kDa	R.f	R.f (2)	R.f (2)
*glt*A	ND	neg	ND
Serology			
Acute phase/s			
Ric-MIF IgG	<64	<64	<64
Ric-MIF IgM	256	128	128
Convalescent phase/s			
Ric-MIF IgG	128	NA	NA
Ric-MIF IgM	1024	NA	NA
Borrelia (IU/ml)			
Serum	neg	neg	neg
(Index) CSF	neg	neg	neg
TBE serology	ND	neg	ND
Herpes serology	neg	neg	neg
Serology enterovirus	neg	ND	ND
Brain CT	Normal	ND	Normal
Outcome	Restored	Restored	Restored

AV, antiviral; DC, doxycycline; WBC, white blood cell; CSF, cerebrospinal fluid; P, penicillin; PCR, polymerase chain reaction; Ric, rickettsia; seq-id, sequence identity; TBE, tick borne encephalitis; R.f, *R. felis*; ND, not done; NA, not applicable; pos, positive; neg, negative; MIF, microimmunofluorescence.

Study 2 (prospective)

The laboratory results of the consecutive patients are summarized in Table 3. Two (P2, P14) of 20 patients with facial palsy and 8/37 patients (P22, P23, P28, P29, P30, P33, P38, P44) with SD presented a fourfold rise in IgG antibody titres judged as confirmative of infection. However, concerning P22 and P23, on the grounds that 23 (P22) and 20 (P23) weeks had passed between S1 and S2, it was assumed that the antibody levels peaked already 3–4 months earlier and were in fact on the way down in the latter assay (S2). Two of the patients (P12, P15) with FNP had serological findings with IgG titres ≥256 indicating a recent or current infection and judged as probable cases and one of the patients with SD (P41) showed a twofold rise in IgG titre and a fourfold rise in IgM titre suggestive of infection. Four of 20 and eight of 37, respectively, were seronegative to *Rickettsia* spp., whilst 12/20 and 20/37, respectively, were seroreactive with IgG and/or IgM antibodies equal to or higher than the cut-off dilution of 1:64. About half of the seroreactive patients only had IgM between 1:64 and 1:128 and were non-reactive for IgG, perhaps as a result of non-specific cross-reactivity due to exposure to other organisms, autoimmune responses or possibly as a sign of a previous exposure. Of all the 57 patients analysed for acute and convalescent sera only one (P25) had IgG antibodies against *Borrelia* spp. Other symptoms such as fever and/or headache occurred, but skin manifestations or swollen lymph nodes were not seen.

Western blot for patients P12, P23, P29 and P44 showed a specific response in the 120–150 kDa region for IgG to whole-cell antigen of *R. helvetica* (Fig. 1). Absorption with *R. helvetica* antigen reduced the dilutions in IFA for serum P23 from 1:512 to <1:64, and the end-point dilutions for detection in WB with whole-cell antigen was reduced from 1:1600 to <1:400 (Fig. 1). Negative controls in the form of serum from healthy blood donors showed no specific reactions.

Table 3 Study 2 (prospective) results of serology for paired sera (S1, S2) for the *Rickettsia* spp. seroreactive patients and for *Borrelia* spp.

No.	Age/ sex	Duration of symptoms (days)	S1		S2		Weeks/S1–S2	Borrelias IgG/IgM (S1/S2)	Treatment	Tick bite
			IgG	IgM	IgG	IgM				
Facial palsy										
P1	51/F	24	<64	512	64	256	20	neg/neg	P	ND
P2	53/F	6	64	128	256	64	6	neg/neg	P, DC	ND
P3	47/M	4	<64	<64	64	<64	20	ND/neg	P	ND
P4	68/F	3	<64	<64	64	<64	5	neg/neg	LT	ND
P5	55/F	5	64	128	NA	NA		neg/neg	P, AV	N
P6	57/F	4	<64	64	<64	<64	4	neg/neg	P	ND
P7	78/F	3	<64	64	<64	<64	7	neg/neg	LT	ND
P8	23/F	5	64	512	64	256	4	neg/neg	P, DC	ND
P9	55/F	3	<64	256	<64	256	3	neg/neg	LT	ND
P10	30/F	4	64	256	NA	NA		ND/neg	P	ND
P11	60/F	5	64	256	<64	128	4	neg/neg	DC	ND
P12	35/F	5	256	512	256	256	6	ND/neg	P, DC	ND
P13	46/F	3	<64	<64	<64	128	7	ND/neg	P	ND
P14	39/M	3	<64	64	128	256	5	ND/neg	DC	ND
P15	47/M	16	256	<64	128	64	5	neg/neg	P	ND
P16	19/M	3	<64	128	NA	NA		ND/neg	P	ND
Sudden deafness										
P17	65/F	4	<64	128	64	128	3	neg/neg	P, DC	ND
P18	66/F	5	<64	512	64	128	4	neg/neg	DC	ND
P19	57/M	3	<64	<64	64	<64	4	neg/neg	P	ND
P20	47/M	4	<64	128	<64	128	22	neg/neg	P	ND
P21	41/F	4	<64	128	NA	NA		neg/neg		ND
P22	52/M	3	<64	<64	256	128	23	neg/neg	P, AV	ND
P23	72/M	2	<64	256	512	512	20	ND/neg	P,	ND
P24	52/M	6	<64	128	NA	NA		neg/neg	P, AV	ND
P25	74/F	8	<64	256	NA	NA		G+/M−/neg		ND
P26	37/M	2	<64	128	<64	64	7	neg/neg	DC	ND
P27	35/M	1	<64	256	64	64	9	ND/neg		ND
P28	72/M	2	<64	256	128	128	5	neg/neg	P, DC	ND
P29	52/M	16	64	64	256	<64	9	neg/neg	P	ND
P30	63/M	3	<64	256	128	128	10	neg/neg	P, DC	ND
P31	31/M	6	<64	64	<64	<64	4	neg/neg	P	ND
P32	70/M	3	<64	<64	64	<64	5	neg/neg		ND
P33	69/M	4	<64	<64	128	128	5	neg/neg	P	ND
P34	46/M	2	<64	<64	64	64	24	neg/neg	P, AV	ND
P35	51/M	320	<64	<64	<64	128	10	neg/neg	P	ND
P36	68/F	6	<64	<64	64	<64	5	neg/neg		ND
P37	81/M	25	<64	128	<64	128	4	neg(S2)		ND
P38	82/F	8	<64	<64	128	128	9	neg/neg	P, DC	ND
P39	74/M	6	<64	<64	64	64	5	neg/neg	AV	ND
P40	43/F	4	<64	<64	<64	128	12	neg/neg	P	ND
P41	68/M	4	64	<64	128	256	7	neg/neg		ND
P42	63/F	3	64	128	64	128	4	neg/neg		ND
P43	45/F	4	<64	<64	<64	256	6	neg/neg	P	ND
P44	71/M	5	<64	<64	512	512	11	neg/neg	P	ND
P45	57/M	3	<64	<64	<64	256	11	neg/neg	P, AV	ND

ND, not done; P1–P45, prospective patient number 1 to 45. P, penicillin; DC, doxycycline; LT, local treatment; AV, antiviral; neg, negative.

Discussion

The present study shows that between 10% and 20% (CI 3.3–44.3) of patients with peripheral facial palsy (Bell's palsy) and between 9.9% and 24.3% (CI 2.6–41.6) of patients with SD presented serological and/or molecular evidence of an underlying rickettsial infection as the cause of cranial neuritis. In four cases the serological specificity was demonstrated by WB, and in three other patients rickettsial DNA was detected in the CSF, where sequencing and analyses of the obtained amplicons showed 100% similarity with the

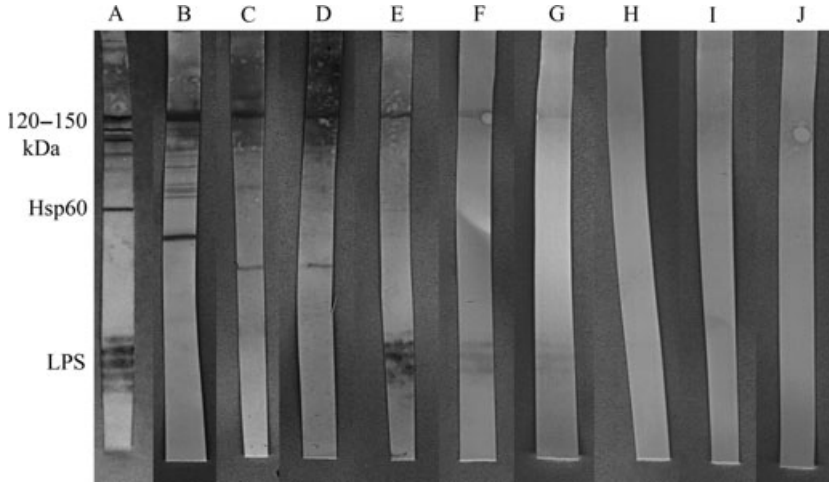

Figure 1 Western blot analysis of IgG antibodies against *Rickettsia helvetica* whole-cell antigen. Lane A demonstrates specific proteins and the lipopolysaccharide (LPS) ladders reacting with a polyclonal rabbit antiserum. Lanes B–E demonstrate the specific reactions against *R. helvetica* proteins in the 110–150 kDa region for serum P29 (B), P12 = (C), P44 = (D) and P23 = (E) in dilutions 1:500. In lanes F–H step-by-step weaker specific reactions to both protein and LPS antigens are shown in dilutions 1:600 (F) and 1:1200 (G) for serum P23 until the reaction has vanished in dilution 1:2400 (H). Lanes I and J demonstrate serum P23 in dilutions 1:600 and 1:1200, respectively, where the antigen−antibody reaction was eliminated after previous absorption with *R. helvetica* whole-cell antigen.

deposited sequence of *R. felis*. Moreover, our study shows that neither FNP nor SD, in any of the cases where CSF was available, was associated with neuroborreliosis and that only two of the 127 patients had antibodies to *Borrelia* spp.

The cause of peripheral facial palsy is unknown in 70% of cases. FNP as an isolated cranial neuropathy has been reported to occur during LB or to accompany acute lymphocytic meningitis and, in some studies, has been demonstrated to account for as much as 65% of acute facial palsy during childhood [25,26]. In previous studies, a Lyme infection has been diagnosed in approximately 10%–20% of patients in Sweden with facial palsy [27] and 10% of adults in Norway [28]. A lower rate of FNP associated with meningitis has been observed during winter indicating that, in these cases, FNP may be caused by other agents [29]. In the rickettsial-DNA-positive patients in Study 1, a slight pleocytosis (0–13 mononuclear cells/l) was found in the CSF, with otherwise normal laboratory values. None of these patients were co-infected with LB. In both Study 1 and Study 2 there was a relatively even distribution of onset of illness in the summer or winter months amongst patients, and of the PCR-positive patients all had become ill between August and February.

There have been only a few reports of isolated facial nerve palsies caused by SFR, in these cases with *R. conorii* as the agent [3,10]. In one case *R. felis* was reported to cause bilateral hearing loss and in two other cases photophobia and signs of meningismus were found [30]. These previously reported patients exhibited low white blood cell counts in the blood and CSF, and a serologically confirmed Epstein–Barr virus infection was found in one of them. These laboratory findings are in good accordance with the findings from previously reported cases of *R. helvetica* or *R. felis* meningitis, where co-infection with herpes simplex virus type 2 was present in one case [16,17]. It is probable, in those cases, that the co-infection may have reactivated the Epstein–Barr virus infection or herpes simplex virus type 2 infection, but the opposite is conceivable, as is simultaneous infection by both agents. The low white blood cell counts make it difficult to use these values to exclude a rickettsial infection, and the assessment will therefore be dependent on other methods such as serology or PCR. Besides those patients who showed fourfold seroconversion or a significant rise in IgG titre – indicating recent, current or probable infection – a number of patients had IgG titres below the cut-off but presented significant increases in titres of IgM antibodies against *Rickettsia* spp., where the highest observed titre was 1/512. The serological IgM response is likely to be specific when the increase in titre is higher than or equal to 256, but the significance of this response, as well as the question of why IgG antibodies did not develop after some weeks, has to be studied further. Three out of three PCR-positive patients showed only IgM antibodies in the acute serum sample. The difficulty in the acute assessment is to know which of these patients have true infections and which of them have non-specific IgM reactivity. In some of the cases the IgM reactivity was

tested positive in WB with whole-cell antigen suggesting that the IgM response sometimes may be specific. It is possible, with the reservation that only six CSF samples were available in the study, that the combination of serology with PCR of CSF in the early stages may provide better guidance as to the causative agents.

Thus far, only one case of African tick bite fever has been reported that was complicated by subacute neuropathy of the eighth cranial nerve [31]. Direct rickettsial invasion of the nervous system has been described in patients with acute neurological complications, but subacute involvement of rickettsiosis is believed to be secondary to immune-mediated mechanisms caused by widespread endothelial inflammation and progression to a vasculitic process that may affect any organ [32,33].

Patients with neuroborreliosis and facial palsy are usually treated with antibiotics, whilst ambiguous facial palsies without neuroborreliosis are given a high dose of cortisone in the acute phase. Three patients in the retrospective group and 14 patients in the prospective group received antibiotics. The antibiotic treatment was in some cases a result of a primary suspicion of borreliosis which later could not be confirmed. In the prospective group most of the patients who showed high titres against rickettsia or seroconversion were treated with tablet doxycycline (100 mg orally twice a day), continued for 10 days (Tables 1 and 3). In previously documented patients, it has been found that the rickettsial disease might be relatively mild [14]. Treatment with cortisone suppresses the symptoms of inflammation and swelling, but it is reasonable to assume that specific antibiotic treatment and antibiotic protection during cortisone treatment should also be given in cases where a rickettsial infection is judged to have caused the paralysis. Doxycycline is preferred over other tetracyclines for treatment of rickettsial infections, and 10 days of treatment is typical. However, the newer macrolides and fluoroquinolones may also be of interest as a form of therapy [34,35]. Generally, prompt initiation of anti-rickettsial therapy is important and may result in a better outcome. These issues must be studied further, together with studies aimed at elucidating the ecology and epidemiology and determining the actual incidence of *Rickettsia* spp. infection in humans as well as its common clinical signs and symptoms.

Acknowledgements

We thank the staff at the Department of Otorhinolaryngology at Falu Hospital for their help with recruiting and testing the patients. The study was financially supported by grants from Uppsala-Örebro-Regional Research 324 Council (25021), the Center of Clinical Research Dalarna (9028) and Olle Engqvist Byggmästare Stiftelse (11877).

Disclosure of conflict of interests

The authors declare no financial or other conflict of interest.

Ethics approval

The study was reviewed and approved by the Regional Ethical Board in Uppsala, Uppsala University.

References

1. James G. All that palsies is not Bell's. *J R Soc Med* 1996; **89:** 184–187.
2. Chau JK, Lin JR, Atashband S, Irvine RA, Westerberg BD. Systematic review of the evidence for the etiology of adult sudden sensorineural hearing loss. *Laryngoscope* 2010; **120:** 1011–1021. Review.
3. Bitsori M, Galanakis E, Papadakis CE, Sibyrakis S. Facial nerve palsy associated with *Rickettsia conorii* infection. *Arch Dis Child* 2001; **85:** 54–55.
4. Tzavella K, Hatzizisis IS, Vakali A, Mandraveli K, Zioutas D, Alexiou-Daniel S. Severe case of Mediterranean spotted fever in Greece with predominantly neurological features. *J Med Microbiol* 2006; **55:** 341–343.
5. Walker DH, Mattern WD. Rickettsial vasculitis. *Am Heart J* 1980; **100:** 896–906.
6. Baganz M, Dross P, Reinhardt JA. Rocky mountain spotted fever encephalitis: MR findings. *Am J Neuroradiol* 1995; **16:** 919–922.
7. Raoult D, Roux D. Rickettsioses as paradigms of new or emerging infectious diseases. *Clin Microbiol Rev* 1997; **10:** 694–719.
8. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev* 2005; **18:** 719–756.
9. Bleck TB. Central nervous system involvement in rickettsial diseases. *Neurol Clin* 1999; **17:** 801–812.
10. Wallménius K, Pettersson JHO, Jaenson TGT, Nilsson K. Prevalence of *Rickettsia* spp., *Anaplasma phagocytophilum* and *Coxiella burnetii* in adult *Ixodes ricinus* ticks from 29 study areas in central and southern Sweden. *Ticks Tick Borne Dis* 2012; **2:** 100–106.
11. Nilsson K. Septicaemia with *Rickettsia helvetica* in a patient with acute febrile illness, rash and myasthenia. *J Infect* 2009; **58:** 79–82.
12. Nilsson K, Wallménius K, Pahlson C. Coinfection with *Rickettsia helvetica* and herpes virus 2 in a young woman with meningoencephalitis. *Case Rep Infect Dis* 2011; **2011:** 469194.
13. Nilsson K, Elfving K, Pahlson C. *Rickettsia helvetica* in patient with meningitis, 2006. *Emerg Infect Dis* 2010; **16:** 490–492.
14. Lindblom A, Severinson K, Nilsson K. *Rickettsia felis* infection in Sweden: report of two cases with subacute

meningitis and review of the literature. *Scand J Infect Dis* 2010; **42:** 906–909.
15. Stenos J, Graves S, Unsworth N. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group rickettsiae. *Am J Trop Med Hyg* 2005; **73:** 1083–1085.
16. Choi YJ, Lee SH, Park KH, *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.
17. 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 Hyg* 2002; **67:** 166–169.
18. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate synthase gene comparison, a new tool for phylogenetic analysis and its application for the rickettsiae. *Int J Syst Bacteriol* 1997; **47:** 252–261.
19. Fournier PE, Raoult D. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. *J Clin Microbiol* 1996; **34:** 2058–2065.
20. Webb L, Carl M, Malloy DC, *et al*. Detection of murine typhus infection in fleas by using the polymerase chain reaction. *J Clin Microbiol* 1990; **28:** 530–534.
21. Nilsson K, Lindquist O, Liu AJ, Jaenson TG, Friman G, Pahlson C. *Rickettsia helvetica* in *Ixodes ricinus* ticks in Sweden. *J Clin Microbiol* 1999; **37:** 400–403.
22. Lindblom A, Wallménius K, Nordberg M, *et al*. Seroreactivity for spotted fever rickettsiae and co-infections with other tick-borne agents among habitants in central and southern Sweden. *Eur J Clin Microbiol Infect Dis* 2013; **32:** 317–323.
23. 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:** 74–77.
24. Hechemy KE, Anacker RL, Carlo NL, Fox JA, Gaafar HA. Absorption of *Rickettsia rickettsii* antibodies by *Rickettsia rickettsii* antigens in four diagnostic tests. *J Clin Microbiol* 1983; **17:** 443–449.
25. Steere AC. Lyme disease. *N Engl J Med* 2001; **345:** 115–125.
26. Tvettnes D, Oymar K, Natas O. Acute facial nerve palsy in children: how often is it Lyme borreliosis. *Scand J Infect Dis* 2007; **39:** 425–431.
27. Jonsson L, Stiernstedt G, Carlson J, Strömberg A, Sjöberg O, Larsson A. Serum and cerebrospinal fluid examination in the diagnosis of Borrelia infection in Bell ′s palsy. *Acta Otolaryngol* 1990; **110:** 421–426.
28. Ljöstad U, Okstad S, Topstad T, Mygland A, Monstad P. Acute peripheral facial palsy in adults. *J Neurol* 2005; **252:** 672–676.
29. Singhi P, Jain V. Bells palsy in children. *Semin Pediatr Neurol* 2003; **10:** 289–297.
30. Zavala-Velázquez JE, Ruiz-Sosa JA, Sánchez-Elias SA, Becerra-Carmona G, Walker DH. *Rickettsiia felis* rickettsiosis in Yucatan. *Lancet* 2000; **356:** 1079–1080.
31. Jensenius M, Fournier PE, Fladby T, *et al*. Sub-acute neuropathy in patients with African tick bite fever. *Scand J Infect Dis* 2006; **38:** 114–118.
32. Marrie TJ, Raoult D. Rickettsial infections of the central nervous system. *Semin Neurol* 1992; **12:** 213–224.
33. Walker DH, Parks FM, Betz TG, Taylor JP, Muehlberger JW. Histopathology and immunohistologic demonstration of the distribution of *Rickettsia typhi* in fatal murine typhus. *Am J Clin Pathol* 1989; **91:** 720–724.
34. Rovery C, Raoult D. Mediterranean spotted fever. *Infect Dis Clin North Am* 2008; **22:** 515–530.
35. Dupont H, Raoult D. Q fever. *Infect Dis Clin North Am* 2008; **22:** 505–514.

