

***Bartonella* species as a cause of infective endocarditis in the UK**

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SUMMARY

Bartonella spp. are increasingly implicated in infectious endocarditis cases in the UK. Herein, we attempted to quantify their role in this syndrome and explored the epidemiology of *Bartonella*-associated endocarditis in the UK. Between November 2005 and October 2010, samples from 685 endocarditis patients were submitted to the Health Protection Agency for *Bartonella* serology. Serological evidence of infection was obtained for 57 (8·3%) patients. PCR-based evidence of infection was obtained from 13 out of 14 patients for whom heart valve tissue was available, with *Bartonella quintana* implicated in 12 cases and *B. henselae* in one. Six patients with *B. quintana* endocarditis were recent immigrants into the UK, of whom four lived in poor socioeconomic conditions. These results indicate that *Bartonella* is a not uncommon cause of endocarditis in the UK and should be considered particularly in patients raised in eastern Europe and/or with a history of homelessness or alcoholism.

Key words: Bacterial infections, bacterial typing, emerging infections, epidemiology, molecular epidemiology.

INTRODUCTION

Bartonellae are small, vector-borne, facultative intracellular, fastidious, Gram-negative bacteria, which are difficult to isolate from humans by routine laboratory methods [1–3]. Several studies on *Bartonella* endocarditis have previously been published, reporting prevalences of 0–4·5% of all infective endocarditis (IE) [4–8]. In France, *Bartonella* spp. are reported to

be responsible for 3–4·5% of IE cases [4–6], while in the UK, a recent study reported that *Bartonella* spp. accounted for 1·1% of IE [7].

To date, seven *Bartonella* species, *B. elizabethae*, *B. alsatica*, *B. koehlerae*, *B. quintana*, *B. henselae*, *B. vinsonii* subsp. *berkhoffii* and *B. mayotimonensis*, have been recognized as the causative agents in blood culture-negative endocarditis (BCNE) in humans [4, 5, 9–13] with *B. quintana* most frequently implicated, followed by *B. henselae*. *B. quintana* was first recognized as a human pathogen during World War I when it was said to be responsible for ‘a greater amount of sickness than any other infection on the

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Western Front' [14]. More recently, *B. quintana* infections have been most frequently encountered in the form of 'urban trench fever' a syndrome afflicting those with a history of poor socioeconomic conditions, especially the homeless and/or alcoholics [15, 16]. The common epidemiological feature of historic and contemporary *B. quintana* infections is infestation by the human body louse (*Pediculus humanus humanus*), which serves as a reservoir and vector for the pathogen [14]. *B. henselae* endocarditis is associated with contact with cats or their fleas and previous valvulopathy [16].

BCNE remains a diagnostic and therapeutic problem, accounting for 12% of IE cases in the UK [7]. While previous antimicrobial therapy is the most common explanation for BCNE [3, 17], the failure to investigate for organisms such as *Bartonella* is thought to account for significant under-ascertainment [6].

The UK's Health Protection Agency (HPA) offers a routine serology-based diagnostic service for human infections caused by *B. henselae* and *B. quintana*. In this paper, we report the results of a 5-year study based on submissions to this service that aimed to better evaluate the prevalence of *Bartonella* infections in IE cases in the UK. Follow-up of serologically confirmed cases also allowed us to use molecular methods to determine the *Bartonella* species involved in these cases and to identify epidemiological associations with the specific *Bartonella* species implicated.

PATIENTS AND METHODS

Patients

Since 1997, the Respiratory and Systemic Infection Laboratory (RSIL), within the HPA Microbiology Services Division – Colindale (London, UK), has offered a serology-based service for diagnosis of suspected *Bartonella* infections in humans. Sera are submitted to RSIL mainly from England but also from Scotland, Wales, Northern Ireland and the Republic of Ireland. Between November 2005 and October 2010 we instigated a follow-up study by requesting clinical material suitable for use in polymerase chain reaction (PCR) from patients for whom we obtained serological evidence of ongoing or recent bartonellosis.

Serology

All sera were examined for the presence of *B. henselae* and *B. quintana* antibodies using *Bartonella* IgG and

IgM indirect immunofluorescent antibody test kits (IFAT; Focus Diagnostics, USA) according to the manufacturer's instructions. Sera were examined in the IFAT at a screening dilution (1:20 for IgM, 1:64 for IgG), and any found to be positive were re-tested in doubling dilutions (to 1:160 for IgM, to 1:512 for IgG) to determine their endpoint: samples still strongly positive at the highest dilution were recorded as >160 or >512, respectively. Based on earlier specificity studies [18] the manufacturer's criteria were adopted for the interpretation of serological results, but with slight modification. Thus an IgM titre of >20 was considered as evidence of current or recent infection, an IgG titre of ≥ 256 as presumptive evidence of recent infection, a single IgG titre of 128 as evidence of infection at an undetermined time, and stable or falling IgG titres from 128 in two sera, taken more than 10 days apart, as suggestive of past infection. An IgM titre of 20 or an IgG titre of 64 was considered as 'equivocal' and a further sample was requested. For all patients for whom an IgM titre of >20 or an IgG titre of >64 was obtained, a request was made on the RSIL laboratory results report asking for any available clinical material (other than blood) to be submitted to RSIL to allow us to undertake further testing using molecular detection methods.

Molecular detection

DNA was extracted from clinical material using the QIAamp DNA mini-kit (Qiagen, Germany) at the HPA Microbiology Services Division – Colindale in London. Extracts were typically prepared in batches of between one and three samples. A water-only negative extraction control was concurrently prepared with each batch. DNA extracts and extraction controls were sent to the University of Liverpool for PCR analysis. The presence and identity of DNA from *Bartonella* spp. was determined using a previously described semi-nested PCR targeting a fragment of the 16S/23S rDNA intergenic spacer region [19] followed by DNA sequencing of amplification products. PCRs were performed on a *ad hoc* basis during the course of the study period, with, typically, samples being tested individually in the presence of only controls. First- and second-round PCRs were performed in purpose-built, dedicated laboratories that were remote from that where amplification products were prepared for sequencing. A positive control, comprising of *B. bacilliformis* DNA, and a water-only reagent control, were concurrently tested

with each batch of samples/extraction controls. The nucleotide sequences of both strands of each amplification product were determined using a commercial sequencing service, with reactions incorporating the same primers as used in the second round of the PCR. Nucleotide sequences were analysed and verified using Chromas Pro v. 1.4.1 (Technelysium Pty Ltd, Australia). Alignment of verified sequences was performed using MEGA v. 4.0 [20].

Patient details

Details, as recorded on the RSIL specimen request form that accompanied the serum and tissue samples, were coded and recorded in the laboratory information management system prospectively, and were analysed retrospectively. Patient age and gender, together with other relevant clinical details and/or history, were noted. These data were occasionally supplemented with information provided, as part of the diagnostic assessment, by the submitting microbiologist.

RESULTS

During the 5-year study period 16 382 sera from 15 417 patients were submitted to RSIL for *Bartonella* serology. Review of the specimen request forms showed that 'endocarditis', 'subacute bacterial endocarditis' or 'culture-negative endocarditis', was recorded as a clinical feature for 685/15417 (4.4%) of the patients. A single serum sample was submitted from 648 of these patients, and two or more sera from 37.

Evidence of current or recent infection (i.e. IgM >20) was found in four cases and presumptive evidence of recent or current infection (i.e. IgG >128) in 23 cases, thus 27/685 [3.9%, 95% confidence interval (CI) 2.6–5.7] of patients with clinical signs of endocarditis had serological evidence of current or recent bartonellosis. Nineteen of the 27 patients were male and the mean age was 51 years (range 18–84 years). Evidence of infection at an undetermined time (i.e. IgG titre of 128) was found in a further 30 cases giving an overall seropositivity rate of 8.3% (57/685) in this series.

Clinical material was requested from all 57 seropositive patients and was received from 14 (24.1%), all of whom had evidence of current or recent infection (Table 1). *Bartonella* spp. DNA was detected in nucleic acid extracts prepared from heart valve tissue of 13 patients. The nucleotide base sequences of both

strands of amplification products were obtained for all 13 samples, and assembly and comparative analysis of these sequences revealed that 12 were derived from *B. quintana* (351 bp, 100% sequence similarity with type strain) and one from *B. henselae* (422 bp, 100% sequence similarity with type strain).

For two patients more than one clinical specimen was received. Three samples were received from patient no. 8, including tissue from the aortic valve, the mitral valve sample and the brain (Table 1). Only tissues from the heart valves yielded PCR products, and the sequences of these products were indistinguishable from one another (*B. quintana*). Indistinguishable sequences were also obtained for amplification products derived from three tissues (aortic valve, mitral valve and left ventricle vegetation) from patient no. 9 (again *B. quintana*) (Table 1).

Of the 13 PCR-positive patients, sera from five had detectable IgM. Of these, one serum reacted against both *B. quintana* and *B. henselae* antigens, three against *B. quintana* alone, and one against *B. henselae* alone (molecular diagnostics on this patient indicated a *B. quintana* infection). Sera from 11 patients had very high IgG titres (>512), 11 against both *B. henselae* and *B. quintana* and one against *B. henselae* only (subsequently found to be *B. henselae* positive by PCR). The aortic valve was affected in seven of the 13 patients, the mitral valve in three, and both valves in one *B. quintana* case and the one *B. henselae* case. The involved valve(s) were unknown for one case. The mean age of the patients in this series was 42 years with a predominance of male patients (67%). Six patients were known to be recent immigrants into the UK, four of whom were reported to have lived in poor socioeconomic conditions either once they arrived in the UK or in the country of their origin.

DISCUSSION

Bartonella spp. are now recognized as a significant cause of human infection. Although a wide range of clinical manifestations of bartonellosis have been described the two most frequently seen syndromes are cat-scratch disease, a zoonotic infection usually caused by *B. henselae*, and IE, which can be zoonotic in origin (e.g. caused by *B. henselae*) but is most frequently caused by the human-restricted *Bartonella* sp., *B. quintana*. In the literature estimates of the frequency of IE caused by *Bartonella* spp. vary from 0–4.5% [4–8]. To date, only one major study of IE in the UK has been performed and this found that

Table 1. Clinical and diagnostic data for the 14 patients from whom tissue was submitted for PCR

Patient no.	Sample date	Age/sex	Valve tissue examined	IFAT results*				Species detected by PCR	Comments
				<i>Bh</i> IgM	<i>Bh</i> IgG	<i>Bq</i> IgM	<i>Bq</i> IgG		
1	Jan. 2006	39 F	Aortic	<20	>512	<20	>512	<i>B. quintana</i>	Recent immigrant from Russia
2	6 Feb. 2006	35 M	Aortic	<20	>512	20	>512	<i>B. quintana</i>	Recent immigrant from Russia: itinerant builder [23]
3	22 Feb. 2006	56 M	Aortic	<20	>512	80	>512	<i>B. quintana</i>	No known risk factors
4	16 May 2006	40 M	Valve†	<20	>512	<20	>512	<i>B. quintana</i>	No known risk factors
5	11 Feb. 2007	39 M	Aortic	<20	>512	<20	>512	<i>B. quintana</i>	Recent immigrant from Lithuania: handyman, known to have lived 'rough' for a few months
6	19 Sept. 2007	58 F	Aortic	<20	256	<20	256	<i>B. quintana</i>	No known risk factors
7	8 Sept. 2007	33 M	Aortic	<20	512	<20	512	<i>B. quintana</i>	Recent immigrant from Czech Republic
8	11 Feb. 2008	18 M	Aortic & mitral‡	80	>512	<20	>512	<i>B. quintana</i>	Recent immigrant from Iran: history of congenital heart disease
9	23 June 2008	36 M	Aortic & mitral	<20	>512	<20	64	<i>B. henselae</i>	No known risk factors [24]
10	22 July 2008	30 M	Mitral	<20	>512	40	>512	<i>B. quintana</i>	No known risk factors
11	1 Sept. 2009	61 F	Aortic	<20	>512	<20	>512	<i>B. quintana</i>	No known risk factors
12	22 Feb. 2010	34 M	Mitral	80	>512	80	>512	<i>B. quintana</i>	Recent immigrant from Poland, lived in squat, heavy drinker
13	May 2010	69 F	Mitral	<20	>512	<20	>512	<i>B. quintana</i>	No known risk factors
14	11 May 2007	62 M	Heart material§	<20	>512	<20	>512	Not detected	Had BCNE and severe pneumonia

Bh, *B. henselae*; *Bq*, *B. quintana*.

* Titres are given as the highest serum dilution yielding a positive result except: <20=a negative result at the screening dilution of 1:20, >512=strongly positive at the highest dilution tested (1:512).

† Recorded as 'heart valve'.

‡ Also a sample of brain tissue, which was negative for *Bartonella* spp.

§ Heart tissue taken from the left atrium.

Bartonella spp. accounted for 1.1% [7]. Over the past two decades serology has become established as the primary diagnostic method for the diagnosis of infections caused by *B. quintana* and *B. henselae* [6, 18]. However, the methodology is poorly standardized (different species are tested for, different antigen preparation methods are used and different Ig subclasses are assayed) and so significance of positive results is not always clear. In the context of endocarditis it has been claimed that high IgG titres (>800) have a very high positive predictive value (PPV) for *Bartonella* endocarditis [6].

We undertook a 5-year study of bartonellosis in the UK and found serological evidence of bartonellosis in 8.3% of endocarditis patients from whom a serum sample was submitted to us for analysis. In about half (3.9%) of these the serology was 'strongly positive' and so indicative of current or recent infection. It is difficult to make a direct comparison between our data and those found in previous studies because it is likely that there is some bias in sample referral to our laboratory (e.g. we may be more likely to be sent sera from BCNE rather than other IE). Nevertheless these data clearly demonstrate that *Bartonella* infection is a significant cause of IE in the UK. If we assume that most of the sera evaluated in our study come from BCNE then the rate of 3.9% of patients having serological evidence of current or recent bartonellosis is lower than the rate of ~10% reported by Lamas & Eykyn [7]. However, the prevalence we report here is much closer to that found elsewhere in Europe, particularly France and Germany [5–7].

In this study we tried to confirm the serology results by obtaining clinical material for PCR. We received material from only 14 of the 57 seropositive patients, and all of these had serological evidence of current/recent infection. PCR confirmed the serological diagnosis in 13/14 cases, strongly supporting the validity of using serology to diagnose *Bartonella* endocarditis. For reasons of economy we did not titrate sera beyond a dilution of 1:512, however in 10/13 PCR proven patients strong fluorescence was seen at this dilution (recorded as >512) thus the endpoint titre must be ≥ 1024 . Although our assay, and the dilution series used, are not the same as those used by Raoult and colleagues [5], our data are in good agreement and strongly support the view that high IgG titres (>800 or >512) have a high PPV. Unfortunately we were not able to assess the significance of weaker serology results (IgG 128) since we did not receive any clinical material for PCR from such patients.

Typically only a few patients have detectable levels of IgM antibodies [18]; however, this subclass is often included in the assay as, unlike IgG, it is thought to allow differentiation between *B. henselae* and *B. quintana* infection. While this was the case for three patients with *B. quintana* infections, in one case IgM against both species was detected and in one case IgM against *B. henselae* was detected but the patient was subsequently shown by PCR to be infected by *B. quintana* (confirmed by repeat testing). Although only a small series, these data do suggest that overall testing for IgM contributes very little to the diagnosis of bartonellosis.

Of the 13 cases confirmed by PCR, 12 were caused by *B. quintana* and one *B. henselae*. While we made no attempt to obtain full patient details, from the available data the clinical features of the *B. quintana* patients were similar to those previously reported [2, 5, 16]; patients were more frequently male, had a mean age of >40 years, and the vegetative lesions were preferentially located on the aortic valve [5]. Although *B. quintana* endocarditis is most often observed in homeless people, often with chronic alcoholism [16], recently cases have been reported in patients with no known risk factors for infection with *B. quintana* [21, 22]. In our series, at least four patients could be described as having classic 'urban trench fever'; however, markedly, we observed that six had a history of recent immigration into the UK, suggesting that *B. quintana* may well be a particularly important cause of endocarditis in UK immigrants, especially eastern European immigrants. For seven patients we were not aware of any known risk factor for bartonellosis. While for some this might simply reflect a lack of information, for two of the female cases (patient nos. 6 and 13) careful enquiry by the local microbiologist did not identify any risk factors. Given that the human body louse is the only known vector for *B. quintana* this presents something of a conundrum and merits further study.

In conclusion *Bartonella* infection should be considered, and serology undertaken, for all patients with endocarditis, especially if there is a history of immigration into the UK from eastern Europe.

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DECLARATION OF INTEREST

None.

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