Epidemiol. Infect. (1999), **123**, 233–240. Printed in the United Kingdom

Serological evidence of *Bartonella* spp. infection in the UK

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(*Accepted 21 June 1999*)

SUMMARY

We reviewed serological and epidemiological data relating to 1000 consecutive patients from whom specimens were submitted for estimation of bartonella antibodies, using MRL Diagnostics Bartonella IFA IgM and IgG kits. Using 289 control sera, we estimated the specificity of the kits as $\geqslant$ 99·0%. Evidence of bartonella infection was found in 16·3% of patients examined. Rates varied by patient group: 20% of patients for whom a diagnosis of cat scratch disease (CSD) was considered probable had evidence of infection, as did 10·4% of patients with 'possible CSD', 8·1% of patients with possible bacillary angiomatosis, 18·2% of patients with 'culture negative' endocarditis and 17·6% of patients with possible bartonellosis with ophthalmic involvement. An IgM response was seen in 6·6% of patients and IgG in 15·1%. Cases were more frequent among males than females (18·5% vs. 13·9%). Analysis by age showed that although rates of infection were highest in the decades 0–9 years (19·4%) and 10–19 years (20·7%), they fell only slightly in the next three decades. MRL bartonella kits appears to provide a useful and specific approach to the diagnosis of these infections.

INTRODUCTION

Following recent taxonomic studies, the genus *Bartonella* now comprises 10 species, 5 of which have been shown to cause infection in humans [1–4]. *Bartonella bacilliformis*, the causative agent of classic bartonellosis (Carrion's disease) has been known since 1909 [5]. However, although cases are occasionally encountered outside the endemic area [6] Carrion's disease is essentially confined to the Andean region of South America and receives little attention elsewhere. *B. quintana* infections were first recognized in the First World War, as trench fever. Although at the time the causative organism could not be isolated, the disease affected thousands of soldiers and was consequently studied in considerable detail. Workers demonstrated that the infection was transmitted by the human body louse and concluded that the causative agent was probably a rickettsia [7]. Although trench fever again became a significant problem during the Second World War, it has been encountered infrequently since [8]. It was not until 1966 that the causative agent was finally isolated [9] by which time the discovery aroused little interest.

It was the application of molecular diagnostic techniques to the study of an AIDS-related disease, bacillary angiomatosis (BA), that led to the identification of *B. henselae* and subsequent renewed interest in the genus *Bartonella*. BA was first recognized in 1983 [10] and although organisms were seen in clinical material, bacteria could not be isolated. Some years later Relman and colleagues applied PCR in a novel way to amplify eubacterial 16S rDNA gene fragments directly from the tissue of four patients with BA. Analysis of the DNA sequences they obtained revealed an organism that was distinct from, but closely related to both *B. quintana* and *B. bacilliformis* [11, 12]. Concurrent work showed that two other AIDS-related conditions, peliosis hepatitis

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and a persistent HIV-associated bacteraemia, were also caused by this organism [13, 14]. Two years later an organism was cultured and isolated from BA patients [15] and confirmed as a new species, *B. henselae* [16]. Soon after, *B. henselae* was implicated as the causative agent of a range of clinical syndromes in the immunocompetent including most notably, cat scratch disease (CSD) [17, 18]. CSD, which is classically seen as a self-limiting but persistent lymphadenopathy in nodes draining the site of a scratch, had been known since the 1950s [19]. However although considered to have a microbiological aetiology the identity of the causative agent had been controversial [17, 20, 21].

It is now clear from a large number of recent studies that *B. henselae* and *B. quintana* cause a wide range of illnesses including CSD, BA and endocarditis [22]. However, to date there are almost no published data regarding the significance of these organisms in the UK. Consequently when commercially produced test kits for the diagnosis of bartonella infections became available in the UK we initiated a study with three aims. These were: (i) to determine the utility of one of these kits (the MRL Diagnostics Bartonella IFA IgM and IgG kits), as an aid to the clinical diagnosis of bartonella infections in the UK; (ii) if the kits proved satisfactory, to make a preliminary estimate of the burden of bartonella infection in the UK, and (iii) to identify epidemiological features of patients with bartonella infections.

METHODS

Patient specimens

All sera from the first 1000 consecutive patients from whom sera were submitted to RSIL for bartonella serology after 1 January 1997 were included in the study. Thus 1128 sera were examined in the period 1 January 1997 to 25 February 1998. Paired or multiple sera were submitted from 102 patients and in these instances second and subsequent sera were always examined in parallel with the early samples.

Other specimens

Sera (one per patient) for specificity studies were kindly made available from the following subjects: blood donors (200), obtained from the North London Blood Transfusion Service; healthy adult contacts (36) of a proven case of *B. quintana* infection; toxoplasmosis patients (16) whose infections had been established by positive IgG titres in the Sabin–Feldman dye test; patients with evidence of current (8) or past (5) mumps virus infection, established by positive IgM or IgG titres respectively; patients giving a history of 'feeling unwell', 'swelling limbs' or 'joint pains' with serological evidence of *Streptococcus pyogenes* infection established by significantly elevated ASOT and/or ADB titres (8); patients with evidence of acute EBV infection established by positive EBVCA IgM titres (11); and patients with a history of contact with psittacine birds and serological evidence of *Chlamydia* spp. infection (5).

Estimation of antibody levels

All sera were examined using both the MRL Diagnostics Bartonella IgG and Bartonella IgM indirect immunofluorescent antibody (IFA) test kits (MRL Diagnostics, Cypress, California) according to the manufacturer's instructions. The IgM kit utilizes blood-agar grown bartonella as antigen, and includes an absorption step to eliminate free or complexed IgG, while the IgG kit utilizes Vero-cell-associated bartonella as antigen. Sera were initially examined at a dilution of 1:20 for IgM or 1:64 for IgG. Any serum found to be positive at this screening dilution was then titrated.

The manufacturer's positive and negative control sera were assayed on every occasion the test was undertaken. In addition, on every occasion before a new batch of kit was brought into use, and periodically thereafter, a known positive serum was titrated beyond its endpoint to ensure that batch-to-batch variation was minimal (i.e. the same endpoint was obtained).

Based on the results of the specificity studies, the manufacturer's criteria were adopted for the interpretation of serological results. Thus an IgM titre of $\geqslant 20$ was considered as evidence of current or recent infection; an IgG titre of $\geqslant 256$ as presumptive evidence of recent infection; a single IgG titre of 64 or 128 as evidence of infection at an undetermined time, and IgG titres of 64 or 128 in two sera, taken more than 10 days apart, as suggestive of past infection.

As part of the laboratory's internal quality assurance (IQA) programme, specimens are selected at random and re-tested blindly. During the period of this study approximately 5% of the specimens received were re-tested and the results of 97% of these were in complete agreement with the original result.

Cross-reaction with chlamydia

Single sera from 11 patients with endocarditis and diagnostic bartonella serology were examined in the Chlamydien-IgM rELISA, -IgA rELISA, -IgG rELISA (Medac GmbH, Hamburg) and the MRL Diagnostics Chlamydia Micro-immunofluorescence (MIF) IgM, IgA and IgG kits (MRL Diagnostics Cypress, California).

Patient details

The details, as recorded on specimen request forms, were reviewed retrospectively. For each patient, age and gender were noted as were presence or absence of the following: lymphadenopathy (including references to 'swollen lymph nodes' or 'painful lymph nodes'); histological evidence consistent with bartonella infection; specific mention of bacillary angiomatosis, peliosis hepatis or HIV infection; 'culture negative' endocarditis or SBE; ophthalmic involvement (e.g. 'scratch to the eye' or Perinaud's oculoglandular syndrome); specific mention of cat contact. If the only details given were of signs or symptoms that had not previously been associated specifically with bartonella infection the patients were scored as 'not appropriate'. Examples include 'arthritis and contact with pets', 'fitting' and 'abscess on breast'. If no details were given, or those given were illegible, the patient was scored as 'no details'. From these data each patient was allocated to a category to reflect the primary reason for which bartonella investigations were requested. Categories were: CSD, bacillary angiomatosis/peliosis (BA), 'culture negative' endocarditis (END), bartonellosis with ophthalmic involvement (OB), 'not appropriate' (NA) or 'no details given' (ND). Those submitted for CSD were subdivided into probable CSD (hereafter referred to as PrCSD) where lymph node involvement or histological evidence were recorded, or possible CSD (hereafter referred to as CAT) where cat contact was the only 'risk' factor indicated.

RESULTS

Specificity studies

The results obtained from the 289 control sera are shown in Table 1. Three sera, from blood donors, were found to be positive with titres of 64 in both the *B. henselae* and *B. quintana* IgG assays. A further two sera were positive at a titre of 20 for *B. quintana* IgM. One of these was EBVCA-positive/EBNA-negative and came from a 21-year-old male with glandular fever and pyrexia. The other was obtained from a 46-year-old male with a 2-week history of lymphadenopathy and a 'flu-like illness, and was positive for both mumps IgM and IgG. No sera were positive for *B. henselae* IgM. Thus, for this series of patients, specificities of the kits ranged from 99% (95% CI 97·0–99·8) to 100% (95% CI 98·7–100·0). The manufacturer's recommended screening dilutions (1:20 for IgM, 1:64 for IgG) and recommended diagnostic criteria (see above) were therefore deemed appropriate for the main study.

Sera from five patients with epidemiological and serological evidence of *C. psittaci* infection all had negative titres in the bartonella IgM and IgG IFA. In contrast the 11 bartonella antibody-positive 'culture-negative' endocarditis patients all gave positive results in one or both, of the chlamydia kits. Thus of the 10 sera examined by MIF, 9 had positive IgG titres (> 64), 3 positive IgA titres (> 16) and 1 had positive IgM titres [10]. Titres were highest against *C. pneumoniae* but in some sera high titres were seen against *C. pneumoniae*, *C. trachomatis* and *C. psittaci*. Of the 7 sera examined using the Medac kit all were IgG positive, 3 IgA positive and 3 IgM positive. Of the 6 sera examined in both assays chlamydia results were in agreement except for 1 Medac IgG positive which was MIF negative and 1 Medac IgM positive which was MIF negative. Attempts were made to 'cross absorb' these sera using the method described by Maurin and colleagues [23] but this was not successful as only partial absorption was achieved. Insufficient sera remained for re-absorption.

Overall serological findings

Sera were submitted from a similar number of males and females (males 482, females 498, gender not known 20) and patient ages ranged from < 1 year to 85 years old.

Serological evidence of bartonella infection was obtained in 163 (16·3%) patients (Table 2). Of these, 108 had evidence of recent infection, 51 evidence suggestive of infection at an undetermined time and 4 evidence of past infection. Within these overall figures the number of patients with evidence of infection ranged from 113 (20·0%) of PrCSD patients to only 5 (4·9%) of NR patients.

Of the 565 patients categorized as PrCSD 110 also had cat contact noted on their request forms: 32

https://doi.org/10.1017/S0950268899002927 Published online by Cambridge University Press

Table 1. *Specificity of the MRL Bartonella IFA IgM and IgG kits determined using sera from 289 control subjects*

	B. henselae				*B. quintana*			
	IgM		IgG		IgM		IgG	
	< 20	> 20	< 64	> 64	< 20	> 20	< 64	> 64
Blood donors	200	0	197	3	200	0	197	3
Contact controls	36	0	36	0	36	0	36	0
Toxoplasmosis	16	0	16	0	16	0	16	0
Mumps virus	13	0	13	0	12	1	13	0
S. pyogenes serology	8	0	8	0	8	0	8	0
EBV	11	0	11	0	10	1	11	0
Chlamydia spp.	5	0	5	0	5	0	5	0
Totals	289	0	286	3	287	2	286	3
Specificity (95% CI)	100·0% (98·7–100%)		99·0% (97·0–99·8%)		99·3% (97·5–99·9%)		99·0% (97·0–99·8%)	

Table 2. *Summary of the combined results obtained using the MRL IFA IgM and IgG kits with sera from 1000 patients*

Patient category (number of patients)	Females (498)				Males (482)				All (1000)			
	R*	U	P	Total	R	U	P	Total	R	U	P	Total
Probable CSD (565)	11·8	5·6		17·4	14·6	7·5	0·8	22·8	13·1	6·5	0·4	20·0
Possible CSD (48)		6·7		6·7	16·7			16·7	6·3	4·2		10·4
BA/Peliosis (37)					9·4			9·4	8·1			8·1
Endocarditis (66)	4·8			4·8	22·0		2·4	24·4	15·2	1·5	1·5	18·2
Ophthalmic involvement (17)		11·1		11·1		16·7		16·7	5·9	11·8		17·6
No details (124)	16·4		1·6	18·0	6·9	10·3		17·2	11·3	5·6	0·8	17·7
Not appropriate (143)		1·5		1·5	4·1	1·4		6·8	2·8	1·4		4·9
Totals	9·4	4·2	0·2	13·9	12·2	5·6	0·6	18·7	10·8	5·1	0·4	16·3

* R, percentage with evidence of recent infection; U, percentage with evidence of infection at an undetermined time; P, percentage with evidence of past infection. 0% are not shown.

Table 3. *Percentage of 1000 patients whose sera gave a positive result against* B. henselae, B. quintana *or both in the MRL IFA IgM and IgG*

Patient category (number of patients)	*B. henselae* only			*B. quintana* only			*Bartonella* spp.			Totals		
	IgM > 20	IgG > 64	Either (M or G)	IgM > 20	IgG > 64	Either (M or G)	IgM > 20	IgG > 64	Either (M or G)	IgM > 20	IgG > 64	Either (M or G)
Probable CSD (565)	6·9	17·9	18·6	1·4	13·1	13·8	0·7	12·2	12·4	7·4	18·8	20·0
Possible CSD (48)	4·2	8·3	8·3	2·1	8·3	10·4		8·3	8·3	6·3	8·3	10·4
BA/Peliosis (37)	5·4	5·4	8·1		5·4	5·4		5·4	5·4	5·4	5·4	8·1
Endocarditis (66)	4·5	12·1	13·6	7·6	15·2	16·7	3·0	12·1	12·1	9·1	15·2	18·2
Ophthalmic involvement (17)	5·9	11·8	11·8		17·6	17·6		11·8	11·8	5·9	17·6	17·6
No details (124)	8·1	16·9	17·7	1·6	9·7	10·5	1·6	9·7	10·5	8·1	16·9	17·7
Not appropriate (143)	1·4	2·8	2·8		2·8	2·8		2·1	2·1	1·4	3·5	3·5
Totals	5·9	14·2	14·9	1·6	10·9	11·6	0·8	10·0	10·2	6·6	15·1	16·3

https://doi.org/10.1017/S0950268899002927 Published online by Cambridge University Press

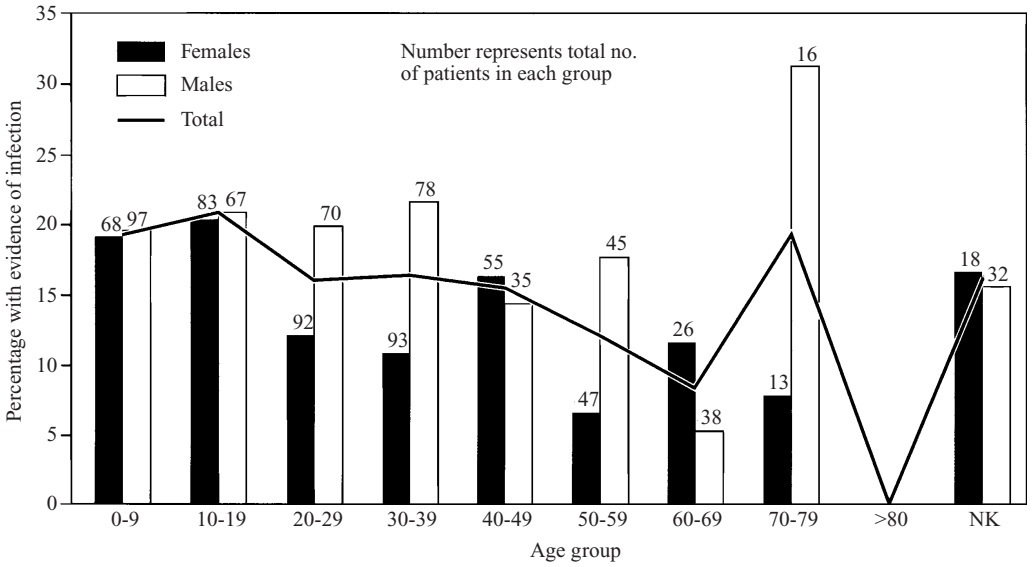

Fig. 1. The age and gender distribution of 163 patients with evidence of bartonella infection.

(29%) of these had evidence of infection (20 recent; 11 undetermined time; 1 past infection). Thirty-two of the PrCSD patients had both lymph node involvement and histological evidence recorded: 17 (53%) of these had evidence of infection. Five PrCSD patients had lymph node involvement, histological evidence and cat contact noted: 4 of these had evidence of infection (2 recent, 2 undetermined time).

Immunoglobulin subclass

The rates of IgM and/or IgG positivity by patient group are shown in Table 3. A positive IgM result was seen in sera from 66/163 (40%) positive patients, 51 of which were *B. henselae* specific, 7 *B. quintana* specific and 8 reacted with both species. In contrast a positive IgG result was obtained for 151/163 (93%) patients of which only 42 were *B. henselae* specific, 9 *B. quintana* specific while 100 reacted against both species.

If only IgG had been estimated for these patients then 12/163 (7·4%, 7 *B. henselae* and 5 *B. quintana*) infections would have been missed completely. A further 11 (6·7%, 10 *B. henselae* and 1 *B. quintana*) would have been considered 'suggestive of infection at an undetermined time' rather than 'recent' infections.

Analysis by patient age and sex

Figure 1 shows the age and gender distribution of the 163 patients with evidence of infection. Overall, more males (18·5%) than females (13·9%) had evidence of infection but the difference was barely significant ($\chi^2 = 3{\cdot}84$, $P = 0{\cdot}05$) and this was also the case for the patient groups PrCSD and END.

Although rates of positivity were highest in the decades 0–9 years (19·4%) and 10–19 years (20·7%), they fell only slightly in the next three (16·0%, 16·3 and 15·4% respectively). These differences were not significant. The highest positivity rate (31·3%) was observed in males aged 70–79 years. This group contained the majority of endocarditis patients.

DISCUSSION

Debré and colleagues first described CSD in 1950 [19] and references to this condition appeared in the UK literature shortly after [24, 25]. Although from the outset the disease was thought to have a microbiological aetiology, workers consistently failed to isolate or identify the causative agent [26]. Diagnosis was therefore determined by the clinical symptoms, a history of cat scratch, failure to identify an alternative cause and most frequently by a positive reaction to a skin test antigen [26, 27]. As the skin test antigen became available in the UK as soon as 1952 [28] CSD became a well recognized condition here. However concerns in the late 1970s about the safety of the skin test antigen led to the cessation of its preparation and distribution in the UK. Thereafter there were very few reports of CSD here [29] although cases continued to be identified in countries such as the USA where use of the skin test antigen continued [27].

After the description of *B. henselae* [16] and its

https://doi.org/10.1017/S0950268899002927 Published online by Cambridge University Press

implication as the causative agent of CSD [17], in common with many other workers [30–33], we developed a range of serological assays for the detection of antibodies against *Bartonella* spp. and utilized these in an attempt to determine the incidence of bartonella infections in the UK [34]. However these early studies were hampered by problems of batch-to-batch variation due to difficulties of in-house production of good quality antigens for the assays. The recent availability of commercially produced (and therefore possibly more consistent) IFA kits offered the possibility of overcoming this limitation, allowing long term serological studies to be undertaken. We undertook this study to determine if the assay was suitable for routine use and to assess the frequency of bartonella infections in the UK.

If a serological assay is to be suitable for the diagnosis of an infection, antibody levels must be detectable in the majority of infected patients at levels that are rarely seen in patients who do not. MRL recommend anti-bartonella titres of 20 and 64 for IgM and IgG respectively, as being significant and our data suggest that these are suitable cut-off levels for the UK population. Although we examined a relatively small number of control sera we found the specificity of both Ig subclasses to be $\geqslant 99\%$ at these cut-off levels. These data are in contrast to those of Zbinden and colleagues [35] who, using the same IgG kit, reported specificities of only 50 or 63% for Swiss urban and urban/rural blood donors respectively. The reasons for these differences are unclear but the data highlight the importance of determining the seroprevalence in each population of interest to ensure that the cut-off levels selected are locally appropriate.

Serum from 1 of the 11 EBV positive patients gave a *B. quintana* IgM titre of 20. Zbinden and colleagues [36] found that sera from 9/20 EBV-VCA IgM-positive patients were also *B. henselae* IgM positive (*B. quintana* antibodies were not determined). As EBV is likely to be one of the main differential diagnoses in patients with possible CSD this finding merits further study. For the present we would suggest that results for a serum that is only *B. quintana* IgM positive should be interpreted with caution, and that EBV serology should also be undertaken.

Serological confusion between the agent responsible for CSD (*Bartonella* spp.) and the 'virus of the lymphogranuloma-psittacosis-trachoma group' (*Chlamydia* spp.) was recognized soon after Debre's first report [37] and has been noted by several workers since [23, 38]. Our data confirm that sera from patients with antibodies against *Bartonella* spp. can also give positive reactions in some chlamydia assays but that the converse does not occur. Unfortunately we were unable to confirm by absorption studies that it was the chlamydia tests that gave the falsely positive results. However this is clearly the case in some instances, as subsequent to this study the diagnosis of bartonella endocarditis was confirmed in 5 of the 11 patients by PCR or culture (author's unpublished results). Maurin and colleagues [23] concluded that these cross-reacting antigens were protein, however we found cross reactions using both the MIF and Medac rELISAs. Since the later assays use recombinant LPS antigens we conclude that the cross-reacting antigens may be either protein or LPS. It seems likely, as reported by others [39] that some cases of 'culture negative endocarditis' diagnosed by serology as chlamydial are in fact caused by *Bartonella* spp.

Given that the IFA kits used appear to be specific for bartonellae the results of this study provide clear serological evidence of bartonella infection in the UK with about 16% of patients investigated having evidence of infection. In the probable CSD group (PrCSD) evidence of infection was demonstrated in 20% of patients. This figure is somewhat lower than that reported in similar studies undertaken in other countries [40]. However this may be simply a reflection of the criteria used to identify patients for testing rather than due to any real difference in rates of infection. In support of this latter view, the data for those patients with good clinical indications of CSD show that 29–53% had evidence of infection whereas fewer than 5% of those whose symptoms were considered 'not appropriate' had evidence of infection.

While it is not possible to determine the infecting species with any confidence by serology, the IgM response is reported by the kit manufacturers to be species specific. In the sera from the PrCSD group of patients most IgM reactivity was seen against *B. henselae*. This observation is consistent with evidence from other countries that *B. henselae* is the primary cause of CSD [17, 41]. Traditionally CSD has been considered to be predominantly an infection of children and young adults [27, 42]. However more recent studies have not confirmed this view [43, 44]. We too found no clear differences in the age specific rates of infection in the first five decades of life. It may be that the earlier studies were biased because they were undertaken predominantly by paediatricians [27].

Although mild self-limiting *B. quintana* infection has been recognised since the First World War it is only recently that life-threatening infection such as endocarditis has been identified [45]. We examined sera from 66 patients with culture-negative endocarditis and evidence of bartonella infection was found in 18% of these. There was clearly considerable ascertainment bias in this sample as colleagues aware of our interest in this area of study sought out sera from such patients to send to us. Nevertheless it seems reasonable to conclude that these data confirm earlier reports that *Bartonella* spp. are an important cause of 'culture-negative' endocarditis [22, 46].

Sera from less than 10% of the HIV and other immunocompromised patients with possible BA examined in this study had evidence of infection. While this could be because few of these patients had BA this seems unlikely as some patients were noted to have histologically confirmed BA. An alternative explanation is that many of them do have BA but do not mount a significant antibody response. Poor antibody responses in this group of patients have been noted by other workers [22]. It seems likely that PCR and similar methods will prove more useful than serology for the diagnosis of such infections.

There is now good serological evidence that *Bartonella* spp. infections are common in the UK and are manifest as CSD, endocarditis and BA. The MRL bartonella kits appear to be useful and specific assays for the diagnosis of these infections although the sensitivity in BA patients is probably quite low. In most instances a diagnosis can be established simply by measuring the IgG response. However if IgM estimation is undertaken this will give a slightly higher yield and may provide some indication of the infecting species. Clearly bartonella antibodies should be determined as part of the differential diagnosis of culture-negative endocarditis as these infections can be fatal if untreated.

ACKNOWLEDGEMENTS

We would like to acknowledge and thank Heather Ford, who expertly examined many of the sera included in this study, and Dr R. C. George for helpful discussions in the preparation of this paper. Also we would like to thank Dr M. Sillis, Dr E. Guy, Mr G. Hutchinson, Ms P. Litton and all our microbiological and clinical colleagues who submitted samples to us for examination. We thank MRL Diagnostics who donated kits for the specificity studies.

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