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Isolation and characterization of *Borrelia burgdorferi* strains from *Ixodes ricinus* ticks in the southern England

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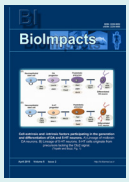

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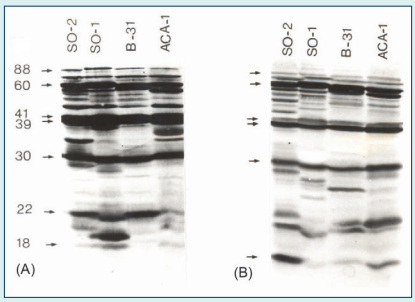
Abstract

Introduction: Lyme disease is a bacterial infection caused by the spiral-shaped bacterium Borrelia burgdorferi. We investigated the presence and prevalence of Borrelia species in ticks from the southern England.

Methods: One hundred fifty-five cases (103 adult and 52 nymphal ticks) were collected from animal carcases. The midguts were removed, cultured in Barbour/Stoenner/Kelly II (BSK-II) and Barbour/Stoenner/Kelly F (BSK-F) media and examined by IF, dark-field microscopy, and nested PCR.



Results: From a total 155 cultured ticks, two showed evidence of spirochetes and denoted as SO-1 and SO-2 strains. The availability of these two isolates enabled their antigenic characterization with SDS-PAGE and western blotting and comparison with two standard isolates. These studies identified six protein antigens with molecular weights of 18, 30, 39, 47, 60 and 88 kDa with particular promise for detecting specific immune responses to *B. burgdorferi* infection including Lyme disease. We also investigated the effect of repeated subculture on the antigenic pattern of UK isolate of *B. burgdorferi*.

Conclusion: As a result of this study, antigenic differences have been seen between the UK isolates and the foreign isolates used as laboratory standards.

Introduction

Lyme disease (LD) is a bacterial infection caused by the spiral-shaped bacterium *Borrelia burgdorferi*.[1] Human becomes infected after being bitten by infected hard-bodied ticks (*Ixodes species*). These ticks (sometimes known as deer, sheep, or woodland ticks) are found in forest and moorland areas and also in suburban parklands; but less frequently found in cone-bearing forests.[2] Areas inhabited by deer are particularly suitable home for ticks, but most ticks do not carry the bacteria and not every tick infested area has a high risk of Lyme borreliosis. The most clinical symptoms associated with the LD include facial palsy, a viral-like meningitis, and other nerve damage or arthritis.[3] All infectious stages respond to antibiotic treatment, but the early stage response is easier, especially when the rash is present. Previous investigations approved the existence of DNA of *B. burgdorferi* in malignant lymphomas has strengthened the hypothesis that infection with *B. burgdorferi* may be causally related to non-Hodgkin lymphoma (NHL) development.[4] Lyme borreliosis areas in the United Kingdom include many sites such as the Exmoor, the Lake District, the Scottish Highlands and Islands, New Forest, North York moors, Thetford Forest, other woodland or heathland areas of the southern England, and the South Downs. Although these areas are high risks for LD, any area where Ixodid ticks are present should be regarded as a potential risk area. It should be mentioned that 50% of diseases acquired in the United Kingdom (UK) are known to have occurred in the southern part of England.[1] The incidence of LD has increased in both North America and Europe including the UK in previous reports. In 2011, the incidence rates of serologically confirmed cases in England and Wales,[5] and in Scotland[6] were 1.73 and 4.36, respectively, per 100,000 total population. Overall, the LD incidence rate in the UK is lower than that in the United States (i.e.,

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7.8) with a total of 24 364 cases in 2011.[7] Nonetheless, LD is becoming an important issue in public health management in the UK as increasing number of people use the countryside for recreation.[8] More than 100 protein species have been detected with sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) of *B. burgdorferi*.[9] Labeling with ^{125}I or biotin has identified 13 major surface proteins with apparent molecular weight of 22, 24, 29, 31, 34, 37, 39, 41, 52, 66, 70, 73, and 93 kDa.[9] The 31 and 34 kDa outer surface proteins (OSPs) are designated OSP-A and OSP-B, respectively.[10] The two outer surface proteins of the North American isolates (OSP-A and OSP-B) are more homogenous than that of the European strains when analyzed by western blotting.[11] In European isolates (especially those from ticks), a major OSP-B is often absent but OSP-A is present in nearly all cultured isolates.[12] Although the existence of Lyme disease in the UK is now well recognized, many problems remain to be resolved.[8] These include knowledge of the distribution of *B. burgdorferi* and the associated risk of contracting infection, the prevalence of anti-*B. burgdorferi* antibodies in apparently healthy individuals living in endemic areas and the development of effective methods of serological diagnosis.[1] In addition, little is known about the characteristics of the strains of *B. burgdorferi* which cause infection in the UK. The aim of this study therefore to investigate the isolation and characterization of *B. burgdorferi* in an endemic area in the south of the UK. Also we investigated the incidence of *B. burgdorferi* infection of *Ixodes ricinus* ticks in the New Forest and surrounding areas.

Material and methods

All materials and reagents were obtained from Sigma-Aldrich Co. LLC (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Antibodies were purchased from KPL (Gaithersburg, MD, USA).

Bacterial strains and growth conditions

All laboratory isolates were grown to reach the late logarithmic phase in Barbour/Stoenner/Kelly (BSK) medium[13] supplemented with 6.7% (vol/vol) heat-inactivated rabbit serum (BSK-II) in tissue culture flasks at 33°C. After subculture, isolates were suspended in BSK-II medium containing 40% (v/v) glycerol and then stored at -135°C. The sources of the laboratory strains of *Borrelia* used in this study are shown in Table 1.

Culture from tick midguts

Ticks were immersed in 70% ethanol for about 30 s and rinsed in sterile PBS for 2 min. After mounting on a paraffin wax plate under a binocular dissection microscope, the tick was cut across the bottom edge using a dissection scalpel. The midgut was cut into two portions and the first was placed into BSK –II medium which was then incubated at 33°C. The culture was examined under dark- field microscopy weekly up to 3 months. The second portion of the midgut was taken for immunofluorescence (IF). Small–sized ticks were immersed in 70% ethanol then homogenized in PBS and diluted to 1 mL with BSK-II. Aliquots were then separately inoculated into the medium. The locations of the ticks which were used for culture is indicated in Table 2.

DNA extraction and nested PCR

Five strains of *B. burgdorferi,* obtained from around the world, were cultured in BSK-II medium. In addition to the standard strains ACA-1 (Sweden) and B-31 (USA), strains of 2B45, 3B56, and 7B49 from Germany were used. The strain of *Borrelia hermsii* (USA), *Escherichia coli* and *Treponema pallidum* provided by Dr. I. Clarke (Southampton) and Dr. C. Penn (Birmingham) were included as controls. DNA extraction and nested PCR were performed as previously described.[14] Two pairs of nested PCR primer sequences were chosen complementary to regions of the *ospA* gene that displayed a high degree of homology with that of the OspB gene.

Antigen preparation

For preparation of *B. burgdorferi* antigen to be used in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), strains were grown in either BSK-II or BSK-F media. At late-log-phase, spirochetes were recovered by centrifugation at 8 000 rpm for 30 min at 4°C, washed 5 times with phosphate buffered saline (PBS) and resuspended in PBS. The antigen preparation was stored in aliquots at -70°C unit immediately before use.

Immunofluorescence (IF)

This method was adapted from a previously published study.[15] The midgut of a tick was suspended in PBS, smeared onto a microscope slide, air dried and fixed in

Table 1. Borrelia strains used in the study

Strain	Isolated from	Location	Source
B31	Tick	New York, USA	Charing Cross Hospital
ACA-1	Human Skin	Stockholm, Sweden	Statens Serum Institute, Copenhagen
2B45	Tick	Berlin, Germany	Hereford PHL
7B49	Tick	Berlin, Germany	Hereford PHL
3B56	Tick	Berlin, Germany	Hereford PHL
B. hermsii	Tick	USA	University of Minnesota
B. persica	Tick	Ardebil, Iran	Pasteur Institute of Iran

Table 2. Locations and numbers of the ticks used for attempted culture of *B. Burgdorferi*

Locations	No. of collected ticks	Adult	Nymph
Savernake Forest (Location A)	60	21	19
Savernake Forest (Location B)	60	17	24
Harewood Forest	65	4	30
Great Ridge (Location A)	80	12	35
Great Ridge (Location B)	75	7	37
Grovely Wood (Location A)	70	22	16
Grovely Wood (Location B)	60	20	11
Cranborne (Location A)	25	6	10
Cranborne (Location B)	25	6	10
Cranborne (Location C)	30	6	10
Ranstone	60	20	15
Eyeworth	60	8	27
Happy Valley	40	13	5
Battery Hill	50	5	23
Fritham	70	13	29
Godshill	50	11	15
Beaulieu	30	13	4
Eyeworth Plain	25	6	-
Lyndhurst	40	17	8
Richmond park	30	-	-

acetone for 10 min. Cultured spirochetes, were used as the control antigen. A sample (15 mL) of a 1:25 dilution of polyclonal anti-*B. burgdorferi* rabbit serum was applied to each slide and incubated in a moist chamber at 37°C for 30 min. The slide was washed 3 times in PBS with stirring for 8 min, rinsed in water and air dried. Then 15 mL (diluted 1:50) of FITC (Fluorescein isothiocyanate) conjugated Goat anti-rabbit IgG (DAKO) was applied and the slide was incubated at 37°C for 30 min. After washing with PBS and mounting in 10% glycerol, the slide was examined by fluoroscopy.

Measurement of protein concentration

Protein concentrations were determined by the colourimetric method of Lowry et al[16] using a commercially available kit (Sigma, USA), as modified by Ohnishi and Barr.[17]

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SDS-PAGE was performed by discontinuous buffer system of Laemmli,[18] with either linear polyacrylamide (12.5%) or gradient gels (10%-25%) as described by Heckels.[19] Proteins were separated electrophoretically at l50 V constant voltage until the blue tracking dye reached the stacking gel/separating gel interface. The voltage was then increased to 200 V and electrophoresis was continued until the dye reached the bottom of the gel. For gradient gels, the proteins were separated electrophoretically at a constant 200 V for l8 h at 4°C. The proteins were stained by PAGE blue[20] or silver staining method.[21]

Western blotting of polyacrylamide gels and immune detection of proteins

The procedure was adapted to that of Towbin et al.[22] The gel was soaked in the blotting buffer for 30 min at room temperature. Six pieces of chromatography paper (Whatman, No. 3), cut slightly larger than the gel, were soaked in blotting buffer (20 mM Tris, 150 mM glycine, 20% methanol, 0.1% SDS (w/v), pH 8.3) and three of them were placed on the anode of a semi-dry electroblotter. On top of these, a sheet of nitrocellulose was placed in order, and followed by the gel and then 3 pieces of chromatography paper. Finally the apparatus was covered by a lid which incorporated the graphite cathode plate. At every stage care was taken to expel all air bubbles. Current was passed at a constant 0.8 mA/cm^2 of gel for l h. At the end of the electrotransfer, the nitrocellulose membrane was rinsed twice in TBS (Tris-Buffered Saline) (20 mM Tris-HCl, 500 mM NaCl, pH 7.4) for 5 min. Membranes were stained with 0.5% (w/v) naphthalene black 12B (BDH, England) in 50% (v/v) methanol and 10% (v/v) acetic acid.

The nitrocellulose was washed five times for 5 min in TBS to remove all traces of SDS and then placed in blocking buffer for 1 h at room temperature which blocks excess binding capacity. Blots were either used immediately or stored at 4°C in blocking buffer (5% (w/v) skimmed milk (Marvel) in TBS). With preparative gels, strips 3 mm wide were cut for reaction with different sera. After washing twice for 5 min in TBS plus 0.05% Tween 20 (TTBS), the blots were shaken for 1 h at room temperature in dilution buffer (1% (w/v) skimmed milk in TBS) containing patients' sera or polyclonal antisera at an appropriate dilution (between 1:100 and 1:1000). Unbound antibodies were washed from the nitrocellulose using four washes of 5 min each in TTBS. The nitrocellulose blot was submerged and shaken gently for 1 h at room temperature in the appropriate alkaline phosphatase conjugated antibody (KPL, USA) diluted 1:3000 in diluent. The blot was then washed three times for 5 min each in TTBS followed by two similar washes in TBS to remove residual tween before the addition of substrate (nitroblue tetrazolium-BCIP [5-bromo-4-chloro-3-indolylphosphate toluidinium salt]). Development was then stopped by washing well with distilled water and the blots were air dried.

Results

Isolation of B. burgdorferi from tick midgut by culture and IF

A total of 103 adult ticks from Savernake Forest, Harewood, Great Ridge, Grovely Wood, and 52 groups of nymphal ticks from all locations (except Eyeworth plain and Richmond Park) were tested using the IF method. The midgut contents of two adult ticks, one from Grovely Wood and one from Savernake Forest, showed positive

IF (Fig. 1) with the polyclonal antiserum but not with the monoclonal antibodies. In addition, one group of macerated nymphal ticks from Battery Hill showed a weak positive result with the polyclonal antiserum.

From a total of 281 inoculated tubes (from 555 cultured ticks), two showed evidence of growth of spirochetes. It is interesting to note that of 555 ticks subjected to culture, only two ticks which produced successful cultures were of the two ticks which showed positive IF. These two strains were designated as SO-1 (isolated from an adult tick, collected from Grovely Wood) and SO-2 (isolated from an adult tick, collected from Savernake Forest).

Prevalence of tick infectivity using PCR

The PCR method was utilized to detect the presence of *B. burgdorferi* in the ticks which had been collected by blanket dragging and also in ticks collected from other countries in which *I. ricinus* had already been identified (Table 3).

SDS-PAGE of total proteins

SDS-PAGE was used to compare strains isolated from different sources.[23] Two UK strains were therefore subjected to SDS-PAGE alongside control strains ACA-1 (Sweden) and B-31 (USA). The protein profile after staining with PAGE Blue is shown in Fig. 2. The gel profiles showed more similarity along with some significant differences, particularly in the major proteins (Table 4). The major differences seen occurred in the region 32- 35 kDa characteristic of the OSP-B. Strain SO-2 had a major band at 35 kDa similar to the presumed OSP-B bands in strains ACA-1 (35 kDa) and B-31 (33 kDa), while strain SO-1 had only a weak band at approximately 32 kDa. Both isolates had a major band at approximately 31 kDa characteristic of the OSP-A, although the band in SO-2 appeared to be slightly lower in molecular weight. Strains SO-1 and SO-2, in common with the vast majority of other strains of *B. burgdorferi,* had major proteins of 4l kDa and 60 kDa

Table 3. Incidence of *B. burgdorferi* infection in ticks determined by nested PCR

Location	No. of ticks tested	No. positive (%)
New Forest		
Beaulieu	5	0
Eyeworth	20	2 (10)
Eyeworth plain	15	0
Fritjam	20	0
Godshill	10	4 (25)
Lyndhurst	15	2 (15)
Inner City Park		
Richmond Park	15	2 (13)
Other areas of Southern England		
Battery Hill	15	2 (13)
Cranborne (Location A)	5	0
Cranborne (Location B)	5	1 (20)
Cranborne (Location C)	5	2 (40)
Great Ridge (Location A)	15	9 (60)
Great Ridge (Location B)	15	9 (60)
Grovely wood (Location A)	15	7 (46)
Grovely wood (Location B)	15	9 (60)
Happy valley	12	1 (8)
Harewood Forest	15	10 (67)
Ranstone	15	2 (13)
Savernake Forest(Location A)	15	8 (53)
Savernake Forest(Location B)	15	8 (53)
Foreign Sources		
Ireland		
Adult	104	35 (33)
Larvae	17	2 (12)
Mice ear	20	1 (5)
Germany		
Adults	88	26 (30)
Iran		
O. tholozani	13	0
I. ricinus	30	15 (50)

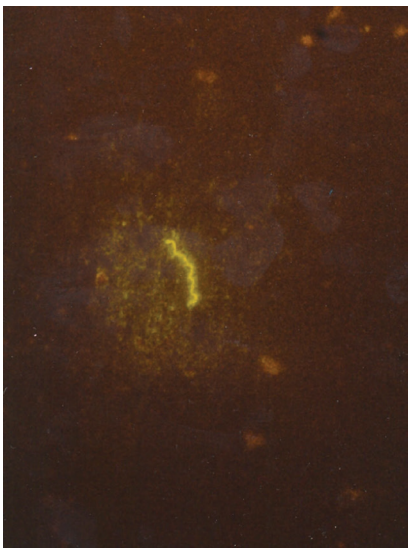

Fig. 1. Immunofluorescence of non-motile spirochetes after attempted culture of contents of tick midgut.

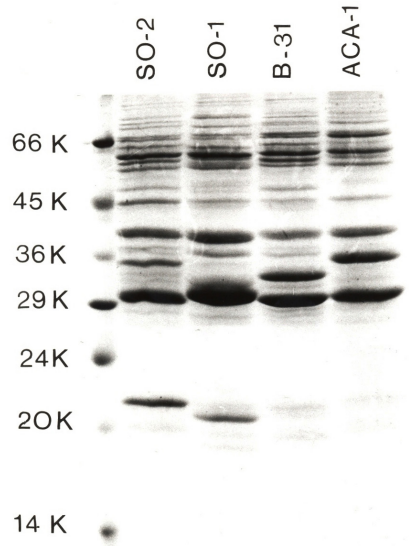

Fig. 2. SDS-PAGE of UK and standard Isolates.

molecular sizes corresponding to flagellin and common antigen, respectively.[24-26] One major difference observed was the presence of a major band at 21-22 kDa in the UK isolates which was not apparent in the standard strains.

Western Blotting

Antigenic heterogeneity among *B. burgdorferi* isolates has previously been demonstrated in isolates from mainland Europe,[12] although North American strains appear to be less heterogeneous.[11] It has been suggested that such differences may correlate with apparent differences in the clinical picture of Lyme disease reported in geographically distinct regions of the world.[12,13] The UK isolates were therefore subjected to western blotting alongside the standard strains in order to detect differences and/or similarities in the antigenic profiles of the strains. The sera used came from UK residents and had previously been shown to exhibit strong reactivity on western blot with the standard strains.

The results shown in Fig. 3 reveal considerable antigenic similarities between the UK and the standard strains. In each case reaction could be seen with the major bands observed on SDS-PAGE (except 31 KDa band), namely the 21/22, 41, and 60/6l kDa bands. One serum reacted with the putative OSP-B- from SO-2 and ACA-1, but not with SO-1 and B-31. The second serum failed to react with any of these bands. In addition, immunological reactivity was also seen with 30 and 39 kDa bands which were not readily detected on SDS-PAGE. Variable reaction was seen with a band of 18 kDa which was only weakly visible on staining of the strains SO-1 and SO-2 and not ACA-1 and B-31.

Monoclonal antibody typing

The western blotting described above demonstrated significant antigenic similarities between the UK and standard isolates. In the attempt to reveal any antigenic differences, the isolates were subjected to serotyping studies with monoclonal antibodies following western blotting.[23] The results are summarized in Table 5.

Table 4. Comparison of major protein profiles of *B. burgdorferi*

Strain	Major bands detected on SDS-PAGE
SO-2	**22**, **31**, **35**, 38, **41**, 45, 55, 58, **60**, 62, 66, 72, 80, 88
SO-1	**21**, **31**, 32, 38, **41**, 45, nd[b], 58, **60**, 62, 66, 72, nd, 88
B-31	nd, **31**, **33**, 38, **41**, 45, 55, 58, **61**, 62, 66, 72, nd, 88
ACA-1	nd, **31**, **35**, nd, **41**, 45, nd, 58, **61**, 62, 66, 72, 80, 88

[a] The strongest bands are shown in bold; apparent molecular weight of major bands obtained from SDS-PAGE gel shown in Fig. 2.
[b] denotes bands which were not detected in individual strains.

The patterns of reactivity obtained showed antigenic differences between two UK isolates. It has been suggested that *B. burgdorferi* strains can be divided into serotypes based on their reactivity with monoclonal antibodies H5332 and H3TS. Type 1 strains react with both antibodies, type 2 strains react with H5332 but not with H3TS, and Type 3 strains react with none. On this basis strain SO-2 is clearly a type 2 strain. However strain SO-1 which reacts with H3TS but not with H5332 cannot be classified by this method. Indeed since this pattern of reactivity has not previously been reported, SO-1 appears to represent a new serological class.

Subcultures of UK isolates

SDS-PAGE and western blotting were carried out on a number of the serial subcultures. The SDS-PAGE profiles and western blotting in one of the sera tested against the isolates have been shown in Figs 4 and 5, respectively. In SDS-PAGE profile the major differences which occurred could be detected as early as passage 3 (P3), and this also appeared similar to the passage 2 shown in Fig. 4. Passage 1 clearly showed the presence of the putative OSP-B protein at 32k Da; this was weakly detectable at passage 3 and further decreased up to passage 10. However at passage 20, a new band appeared at 33 kDa which appeared to shift to 34 kDa by passage 32. In contrast, P1 lacked at 21/22 kDa band which was observed in all other subcultures. Less marked differences were seen with the 41 kDa band which showed increased intensity after

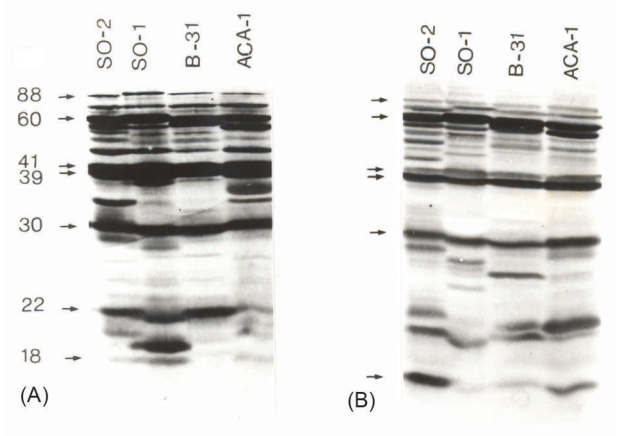

Fig. 3. Western blot of UK and standard isolates with serum from Uk Lyme disease patients. Patient (A) was a New Forest worker who was diagnosed as having Lyme disease and then developed facial palsy. Patient (B) from Leeds was bitten by tick in Scotland.

Table 5. Immunology reactivity of UK isolates with monoclonal antibodies[a]

Antibody	H5332	H3TS	H9724	H605	H6TS	LA31
Antigen recognized	(OSP-A)	(OSP-A)	(41 kDa)	(41 kDa)	(41 kDa)	(OSP-A)
Strain						
SO-1	-	+++	+++	+++	+	+++
SO-2	++	-	+++	+++	++	+++

[a] Monoclonal antibodies H6831(OSP-B), H63(OSP-B), H68(OSP-B), H614(OSP-B), LA2(OSP-A) and LA5(OSP-A) reacted to none of UK strains.

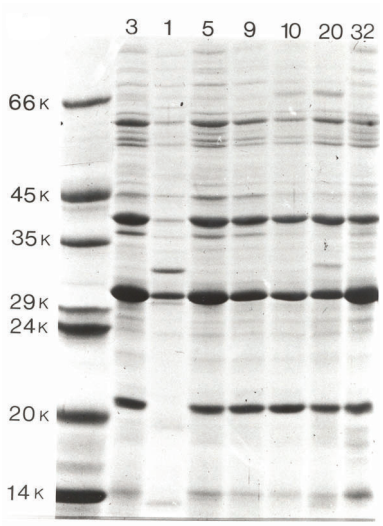

Fig. 4. Effect of serial subculture on protein expression by *B. burdorferi* strain SO-1. Track numbers indicate passage numbers.

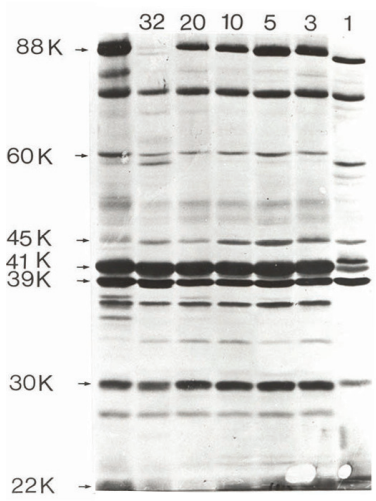

Fig. 5. Western blot of serial subcultures of *B. burdorferi* strain SO-1. Track numbers indicate passage numbers.

P1 while the 45 kDa showed a progressive decrease in intensity with repeated subculture. In western blotting as can be seen in Fig. 5 the intensity of most bands did not change with subculture and the 32 kDa putative OSP-B which was strongly present only in P1 did not react with the serum at all. In contrast some additional bands not present in P1, in particular a band at 36 kDa, was detected in the subsequent subcultures. Thus maximum detection of antibodies could be achieved not with P1, but with the subsequent subcultures.

Discussion

Since the first description of a case of Lyme disease in the UK in 1977, a number of studies have been carried out. The first cases to be reported were from Scotland and East Anglia but since then the majority of cases have been from the South of England[27,28] and elsewhere in the UK[29-31] associated with tick bites experienced in wooded regions. The New Forest area of Hampshire has been particularly associated with the occurrence of Lyme disease. The initial aims of this research were to extend these types of studies to examine the prevalence of Lyme disease and incidence of *B. burgdorferi* infection of *Ixodes ricinus* ticks in the New Forest and surrounding areas. The *B. burgdorferi* is endemic in the New Forest and this was unequivocally confirmed by the use of PCR. The use of oligonucleotide primers corresponding the OSP-A and OSP-B in a "nested PCR" reaction provided a sensitive and specific method of detecting *B. burgdorferi* genome in infected ticks. These studies showed widespread occurrence of infection in ticks collected throughout the area, although large differences were seen in the infection levels in ticks collected from different locations. The PCR reaction was also used to study the prevalence rates in *I. ricinus* ticks collected from Germany, Ireland and Iran with positive reactions of 30%, 33% and 50% of ticks, respectively. The presence of Lyme disease in Germany and Ireland has been well documented,[32] but to date there are no reports of Lyme disease from Iran or other countries in the Middle East although Stanek et al[32] demonstrated that *I. ricinus* is distributed throughout North of Iran. No carriage of *B. burgdorferi* was detected in contrast to the 50% obtained with *I. ricinus* from the same location. The proportion of ticks infected in endemic areas, demonstrated using PCR are comparable to those detected by IF in the East Coast of USA,[33] Eastern Europe,[34] and Asia.[35] There would appear to be a number of possible reasons for the apparently lower incidence of infection in the UK, firstly a failure to recognize the disease in the UK. However Lyme disease is a "high profile" infection in the New Forest, and general practitioners and hospital clinicians are experienced at identifying infection, and with the present levels of public awareness it is unlikely that significantly more cases are being missed there than elsewhere. Further, lower numbers of spirochetes in ticks causing an inoculums effect might affect the course of disease, with regard to the development or non- development of clinical symptoms. The two strains isolated from infected ticks, SO-1 and SO-2, represent the only available UK isolates of *B. burgdorferi.* Two earlier reports represent the isolation of UK strains but in neither case a pure UK isolate was obtained. In one case, the isolate could not be cultured free from a contaminating *Pseudomonas maltophilia.* The strain was isolated as a mixed culture and could be subcultured successfully while contaminated, but failed to grow once purified.[36] In the current study the SO-1 strain was also initially isolated as contaminated culture but could be purified by the use of antibiotics followed by filter enrichment. This strain was isolated and grown in BSK medium which had been modified by replacement of the standard rabbit with fetal calf serum (BSK-F). Other workers have reported the use, in a serological study, of a UK strain which had previously been isolated from a tick taken from a dog by one of the authors.[37] However the details of this isolation have not been published and

the strain is apparently no longer available. The initial characterization carried out on this strain suggested that it was markedly similar in antigenic profile to the US isolate B-31. Although comparison of the two UK strains isolated during the current study (SO-1 and SO-2), with two widely used laboratory strains, ACA-1 from Sweden and B-31 from the USA, demonstrated many similarities as detected by SDS-PAGE and western blotting, significant protein and antigenic differences were observed. It is now evident that the strains of the spirochete can change in expression of proteins and antigenic reactivity when they are serially passaged *in vitro*. Therefore, we investigated the effect of repeated subculture on the antigenic pattern of UK isolate of *B. burgdorferi*.[38-40] For example, we investigated the presence of 21/22 kDa band which was found only in the UK isolates, and the differences in apparent Mr which were observed in both the OSP-B and 60/61 kDa bands. Another major difference was also revealed by the monoclonal antibody typing in which strain SO-2 was shown to be type 2, but strain SO-1 was untypable. Although non-typable strains have not been reported in the literature such strains have occasionally been isolated from Western Europe but not from the USA. Thus it would appear that strain SO-1 more closely resembles European strains.

Conclusion

Since little is known about the determinants of pathogenicity of *B. burgdorferi,* and above UK isolates do not appear atypical when compared with other European isolates, it is not possible to comment on potential pathogenic differences. As a result of the differences observed between the UK isolates and the foreign isolates used as laboratory standards, the UK strains can therefore be used for seroprevalence studies with sera from the New Forest endemic area.

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Ethical issues

There is none to be declared.

Competing interests

The authors declare no conflict of interests.

Research Highlights

What is current knowledge?

√ Lyme disease is becoming an important issue in public health management in the UK.

√ A number of outer surface proteins (OSP) from *B. burgdorferi* have been detected.

What is new here?

√ Two new strains denoted as SO-1 and SO-2 were isolated from cultured ticks.

√ Antigenic differences were observed between the UK isolates and the foreign isolates used as laboratory standards.

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