Notable Cases

Culture-positive Lyme borreliosis

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We report a case of Lyme borreliosis. Culture of skin biopsy was positive for *Borrelia garinii*, despite repeated prior treatment with antibiotics. The patient had travelled in Europe 17 months before the onset of symptoms, but the clinical details indicate that the organism could have been acquired in Australia. The results of conventional serological and histopathological tests were negative, despite an illness duration of at least two years. (MJA 1998; 168: 500-502)

Several reports of possible Lyme disease in New South Wales were published in the 1980s. Attempts to isolate spirochaetes (the causative organism) from ticks have been unsuccessful, but patients with suspected Lyme borreliosis acquired in Australia were shown to be more likely to have antibodies to outer surface protein A (OspA) of one or more of *Borrelia garinii*, *B. afzelii* or *B. burgdorferi* sensu stricto (Box 1) than those whose illness was thought less likely to be Lyme borreliosis.

Clinical record

A 42-year-old man presented to hospital in 1996 with a history of recurrent skin lesions and episodic generalised myalgias and bilateral knee-joint arthralgias (without joint swelling) after a tick bite almost two years previously (Box 2). He also described fatigue; difficulty with concentration, short term memory and thought processes; and a sensation of “fullness in the head”, rather than headache. He had been unable to work for the past eight months.

Examination revealed a faint but definite 30 cm by 20 cm lesion over the right lower chest wall and flank, with a more prominent margin anteriorly than posteriorly, and a suggestion of central clearing (Figure 1). There were no other abnormalities present and no history of relevant previous illness. In particular, there was no facial-nerve weakness, or symptoms suggestive of cardiac involvement, meningitis, radiculitis or neuropathy, the other main clinical manifestations of systemic Lyme borreliosis.

1: Nomenclature

*Borrelia burgdorferi* sensu lato is the name given to a group of organisms including, currently, at least 10 species or genomic groups. The species most commonly recognised as causing Lyme borreliosis are *Borrelia garinii*, *Borrelia afzelii*, and *Borrelia burgdorferi* sensu stricto.

Investigations

- Enzyme-linked immunosorbent assay (ELISA) for antibody response to *B. burgdorferi* sensu stricto gave negative results (Institute of Clinical Pathology and Medical Research, Westmead Hospital).
- Western blotting (Department of Virology, Faculty of Medicine, University of Newcastle) showed 2+ antibodies (one level below the strongest staining intensity) to outer surface protein A (OspA) of *Borrelia garinii* only.
- Skin biopsy showed a mild, mainly perivascular, lymphohistiocytic inflammatory cell infiltrate in the superficial dermis, minimal exocytosis, and a little pigment incontinence; no organisms were demonstrated on periodic acid–Schiff (PAS), Gram or Warthin–Starry silver stains. No diagnosis could be made from the skin biopsy, but differential diagnoses included superficial gyrate erythema and drug eruption.
- Culture of a skin biopsy by standard methods revealed spirochaetes growing after three weeks' incubation.
- Direct immunofluorescence antibody staining of the isolate with fluorescein-labelled antibody to the flagellin protein of *Borrelia burgdorferi* sensu lato (Figure 2) (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA) and polymerase chain reaction amplification of the flagellin and 16S rRNA genes showed that the spirochaetes were *B. garinii* and more closely related to European rather than Asiatic *B. garinii* strains.

The immunofluorescent staining showed clumping, which is typical of initial isolates, as opposed to high-passage, laboratory-adapted strains. Furthermore, no isolates resembling this organism are kept in our laboratory, making it impossible for the isolate to be a laboratory contaminant. Accession numbers for the *B. garinii* flagellin and 16S rRNA sequences are D89899 and D89900, respectively.

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2: History of the tickbite, its sequelae and treatment

Our patient sustained a tickbite on the inner aspect of his left thigh almost two years before hospital presentation. The bite probably occurred while he was walking in bushland in the Pittwater Shire of Sydney. The tick was removed about 24 hours after the bite, and there was no significant lesion at the bite site.

Sixteen days later he developed an erythematous circular lesion, maximum diameter of 5 cm, at the tickbite site. It was not pruritic, but had a prominent margin and central clearing. Associated symptoms included a mild headache, malaise and possibly a low grade fever.

Two days later (18 days after the tickbite), his local general practitioner prescribed a 14-day course of doxycycline (200 mg daily) for possible erythema migrans. Within two days of starting to take the drug, he developed a generalised non-pruritic rash, associated with insomnia, generalised arthralgias, myalgias and possibly fever, although his temperature was not taken. These symptoms, including the rash, settled by Day 7 of treatment. Four days after completing the doxycycline course, he again developed insomnia, as well as difficulty with memory and "thinking clearly". The presumed erythema migrans lesion at the bite site took about three months to resolve completely.

Over 18 months from commencement of the doxycycline, he had recurrent skin lesions: one 5 cm by 10 cm on the right flank, which had first appeared during the doxycycline course, and another on the right thoracic wall that reached about 20 cm in diameter. However, both lesions had fluctuated in intensity and size, often disappearing completely. One week before hospital presentation, he had completed an additional seven-day course of doxycycline (200 mg daily).

Travel history

Our patient was born and raised in Pittwater Shire, residing for only a brief period in inner Sydney. Seventeen months before his symptoms began, he visited three countries in Europe known to be endemic areas for Lyme borreliosis — Italy, Austria and the Czech Republic. He spent three days in Rome, then, travelling by road to Vienna, spent two days in Poprad, on the border of Italy and the Czech Republic. He did not recall any tickbites or any exposure to ticks.

Management

The patient was prescribed amoxicillin (500 mg three times a day), but this was stopped after 14 days because of gastrointestinal upset. He then completed a course of ceftriaxone (2 g intravenously daily for 15 days), to be followed by 12 weekly injections of benzathine penicillin 1.8 g. The skin lesions continued to recur, although they were less extensive, and his other symptoms also recurred after the course of ceftriaxone was finished. They were still present after three weekly benzathine penicillin injections. Thereafter, he was lost to follow-up.

Discussion

Apart from this being the first reported isolation of B. burgdorferi sensu lato (Box 1) from a clinical specimen in Australia, this case illustrates the difficulties involved in diagnosing and treating Lyme borreliosis.

The spirochaete (B. garinii) isolated from this patient may represent the first indigenous clinical isolation of the causative organism of Lyme borreliosis in Australia. However, the history of travel to an endemic region in the northern hemisphere makes it difficult to determine whether the infection was acquired in Europe or Australia. A number of points favour Australian acquisition. The patient was adamant that he sustained no tick bites in Europe, and insisted that he first became unwell after the tickbite in Australia, which preceded the initial skin lesion on his inner thigh. This lesion did not appear until 16 days after the tickbite, distinguishing it from an allergic reaction, and it was observed by his family doctor. According to accepted criteria this initial tickbite lesion is more likely to be erythema migrans.

However, Lyme borreliosis can have a long incubation period, so the 17-month delay between travel to Europe and the onset of skin lesions is not inconsistent with

Figure 1: Torso of patient, showing skin lesion from which a biopsy was obtained. Figure 2: Borrelia garinii isolate from patient, demonstrating clumping typical of initial isolates. Direct immunofluorescence preparation using fluorescein-labelled antibody to B. burgdorferi sensu lato flagellin protein. Magnification x400.
a European acquisition. Many Australians travel to Lyme borreliosis-endemic areas, and some have acquired the disease without being aware of a tick bite. This, coupled with its long incubation period, often makes it difficult to determine conclusively where Lyme borreliosis was acquired.

Although the *B. garinii* isolate is related to European isolates, it may have been introduced into Australia by birds migrating from the northern hemisphere, where it has a wide distribution. Positive polymerase chain reaction data exist to show the presence of *B. garinii* in ticks in the southern hemisphere.⁹ Failure to demonstrate spirochaetes in biopsies of erythema migrans lesions with Warthin–Starry silver stain, as in our patient, is not uncommon, and the histopathological appearance was not characteristic of erythema migrans.

Despite the Centers for Disease Control and Prevention (CDC) case definition,⁸ even patients with late stage Lyme borreliosis may show negative results of serological tests.¹⁰ Our patient’s serological results are of interest. ELISA performed at a reference laboratory gave negative results, but western blotting performed at another laboratory detected antibodies to outer surface protein A of *B. garinii*, which correlated well with the identity of the skin isolate. We have reported previously that antibodies to *B. garinii* outer surface protein A, especially, were most discriminatory in detecting possible Australian cases of Lyme borreliosis.⁹

In previous attempts to detect Lyme spirochaetes in Aus-

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**Figure 3:** Phylogenetic trees constructed by the neighbour-joining method from 16S rRNA gene sequences (A) and flagellin gene sequences (B). Bar = percentage difference between sequences, as determined by measuring the lengths of the horizontal lines connecting species. Source of most closely related isolates: NSB1, tick, Sweden; HT17, tick, Japan; KL10, tick, Czech Republic; G1, patient, Germany; G2, patient, Germany; 20047, tick, France.

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**References**