Case Report

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Percutaneous exposure resulting in laboratory-acquired leptospirosis – a case report

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A screw-capped glass tube containing a Leptospira culture accidentally broke and the laboratory worker who was handling the tube sustained a cut on his hand. The wound was flooded with the culture. The culture was that of strain MG 347 belonging to serovar Australis recovered from a patient, and it had undergone 52 passages in Ellinghausen McCullough Johnson Harris medium. The laboratory worker developed a headache 21 days after the accident and became febrile the next day. He was hospitalized for 5 days and was treated initially with doxycycline and later with ciprofloxacin. A blood sample collected on the second day of illness, after starting doxycycline therapy, yielded leptospires and the isolate, HZ 651, was identified as serovar Australis. Monoclonal antibody patterns and randomly amplified polymorphic DNA fingerprinting patterns of the isolate and strain MG 347 were identical, thus indicating that HZ 651 and MG 347 were clonal.

Introduction

Leptospirosis, caused by pathogenic leptospires, is a direct zoonotic disease (Schwabe, 1984) of worldwide distribution. Human infections are incidental and occur through direct or indirect contact with infected animal urine (Faine et al., 1999). The disease presents as mild febrile illness and subsides without complications in the majority of patients. However, in some patients it progresses rapidly to a severe and fatal form due to multi-organ involvement. The incubation period is variable and ranges from 2 to 20 days (Turner, 1967). Different occupations pose a risk of acquiring leptospiral infections. Veterinary personnel and laboratory workers who deal with animal carriers and live leptospires are among the high-risk groups. Accidental infection during necropsy of animals (Campagnolo et al., 2000) and while handling leptospiral cultures have been reported (Gilks et al., 1988). Leptospirosis is one of the common laboratory-acquired infections (Sullivan et al., 1978).

Case report

On 21 April 2003, while a 27-year-old male laboratory worker was opening a screw-capped glass tube containing a well-grown culture of leptospires in Ellinghausen McCullough Johnson Harris (EMJH) semi-solid medium, the tube broke and he sustained a cut on his hand with the shrapnel. Although he was wearing disposable polythene gloves, the shrapnel cut through the gloves. The cut was flooded with the culture from the broken tube. He cleaned the wound with soap and water and left it open. The culture was an isolate (MG 347) recovered from a patient in the Andaman Islands and was identified as serovar Australis. The culture had undergone 52 passages in EMJH medium (Difco).

On 12 May (21 days after the incident) the worker developed a headache, followed by fever on the next day, and was treated with doxycycline. However, the fever did not subside and he developed vomiting on the same day. He was admitted to a referral hospital on 14 May and blood was collected for investigations on the same day. Doxycycline was discontinued and he was put on ciprofloxacin. On the 5th day after the admission, he recovered and was discharged with advice to continue the antibiotic therapy for another 2 days. A convalescent serum sample was collected on the day of discharge (7th day of illness).

Laboratory findings

The Lepto dipstick assay (IgM immunoassay) (Gussenhoven et al., 1997) and a microscopic agglutination test (MAT) were performed on acute and convalescent sera at our laboratory. The Lepto dipstick assay was performed as per the instructions of the manufacturer (KIT), whereas MAT was performed using nine live leptospiral strains as antigens following standard procedures (Wolff, 1954). The blood sample collected during the acute stage of the disease was inoculated into EMJH semi-solid medium and incubated at 30 °C. The culture was examined at weekly intervals. Blood culture for Salmonella spp. and blood smear examination for malaria parasites were also performed on the blood sample.
collected during the acute stage of the disease. The Widal tube test (Span Diagnostics) was done on both the acute and convalescent samples.

Both the Lepto dipstick and the MAT were negative on the sample collected during the acute stage of the disease. The dipstick was positive and the MAT showed a titre of 1 in 200 against serovar Australis on the convalescent sample. Culture for salmonellae, the Widal test and blood smear examination for malaria parasites were all negative. Renal and liver function tests were normal. Isolation of leptospires was successful after 2 months incubation and the isolate was coded as HZ 651. The isolate was characterized using 36 monoclonal antibodies (KIT) as reported earlier (Sehgal group sera belonging to 23 serogroups and a panel of five coded as HZ 651. The isolate was characterized using 36 monoclonal antibodies (KIT) as reported earlier (Sehgal et al., 2000; Vijayachari et al., 2003). HZ 651 was identified as belonging to serogroup Australis and serovar Australis. The monoclonal antibody patterns of strain MG 347 and isolate HZ 651 were similar (Fig. 1). Randomly amplified polymorphic DNA (RAPD) fingerprinting was carried out as reported by Brown & Levett (1997) on MG 347 and HZ 651 along with 12 reference strains representing eight species: *Leptospira interrogans* serogroup Australis (serovar Australis strain Ballico, serovar Bratislava strain Jez Bratislava), serogroup Icterohaemorrhagiae (serovar Icterohaemorrhagiae strain RGA); *Leptospira kirschneri* serogroup Grippotyphosa (serovar Ratnapura strain Wumalasena, serovar Grippotyphosa strain Moskva V), serogroup Cynopteri (serovar Cynopteri strain 3522C); *Leptospira weilii* serogroup Celledoni (serovar Celledoni strain Celledoni); *Leptospira borgpetersenii* serogroup Javanica (serovar Poi strain Poi), serogroup Mini (serovar Mini strain Sari); *Leptospira santarosai* serogroup Grippotyphosa (serovar Canalzone strain CZ188); *Leptospira noguchii* serogroup Louisiana (serovar Louisiana strain LSU1945); *Leptospira meyeri* serogroup Ranarum (serovar Ranarum strain ICF); *Leptospira biflexa* serogroup Semarangia (serovar Patoc strain Patoc 1). Primer PB-1 (GGGCTTGCTCAG) was used. Analysis of RAPD fingerprints showed that isolate HZ 651 and strain MG 347 were genetically similar (Fig. 2). These strains (MG 347 and HZ 651) showed close relatedness to strains Ballico, Jez Bratislava and RGA, indicating that these probably belonged to species *L. interrogans*.

**Discussion**

Although laboratory-acquired leptospirosis has been well documented in the literature (Campagnolo et al., 2000; Gilks et al., 1988; Sullivan et al., 1978), investigation of such accidents would be helpful in generating new observations. In this report, our focus was mainly on the virulence of laboratory strains, duration of the incubation period, efficacy of doxycycline as a therapeutic agent and usefulness of RAPD as a molecular genetic tool to identify the source of infections.

In spite of all precautions, laboratory accidents occasionally occur. In the present study, the accident occurred due to breakage of the culture tube while handling. Culture tubes are re-used after washing with cleaning fluid (10 % potassium dichromate, 25 % sulfuric acid, 75 % distilled water) and sterilizing in a hot air oven in our laboratory. Perhaps the tube had become brittle after repeated washing and sterilization and broke when the laboratory worker attempted to unscrew the over-tight cap. Although the laboratory personnel working in the leptospira laboratory are instructed to report to medical personnel in the case of accidents (Faine et al., 1999), in the present case this was not observed. The laboratory worker revealed the accident only when he was being interviewed during the clinical examination. He developed a headache as the first symptom on the 21st day after the accident, followed by febrile illness and vomiting on the next day, indicating that the incubation period was 21 days. This is interesting to note as the incubation period for leptospirosis ranges from 2 to 20 days and often it is between 5 and 14 days (Turner, 1967).

Strain MG 347 was originally isolated from a 10-year-old boy from South Andaman who reported to a primary health centre with febrile illness. Since the isolate from the laboratory worker revealed the accident only when he was being interviewed during the clinical examination. He developed a headache as the first symptom on the 21st day after the accident, followed by febrile illness and vomiting on the next day, indicating that the incubation period was 21 days. This is interesting to note as the incubation period for leptospirosis ranges from 2 to 20 days and often it is between 5 and 14 days (Turner, 1967).
monoclonal antibody pattern was similar to that of strain MG 347, it can be inferred that the laboratory worker was infected with the same strain. The RAPD fingerprinting pattern showed 100% genetic similarity between isolate HZ 651 and strain MG 347, further substantiating the findings that both MG 347 and HZ 651 were clonal.

Strain MG 347 retained its pathogenic potential even after 52 passages in EMJH medium. The isolate was recovered from blood culture after more than 2 months incubation. Different laboratories incubate their blood cultures for varying periods ranging from 21 days to 6 months. The isolate could have been missed had the culture been discarded after 3 weeks incubation. Blood culture was attempted after the three doses of doxycycline therapy and isolation of leptospires was successful. In an earlier report, leptospires were isolated from the patient’s urine about 2 weeks after the onset of symptoms when he had recovered from illness following a course of doxycycline (Natarajaseenivasan et al., 2002). Laboratory workers working in leptospira laboratories are at high risk for leptospiral infection as there is a chance of direct exposure to high concentrations of leptospires while handling cultures. A total of three laboratory-acquired leptospirosis cases were reported from the National Animal Disease Center during 1960 to 1976 (Sullivan et al., 1978). Apart from these, a laboratory technician developed leptospirosis following accidental inoculation despite prompt administration of parenteral penicillin (Gilks et al., 1988). Therefore, laboratory workers who deal with live leptospires should be always being investigated for leptospiral infection if they develop acute febrile illness, to initiate specific treatment.

References


