

Detection and characterization of *Leptospira interrogans* isolates from pet rats belonging to a human immunodeficiency virus-positive patient with leptospirosis

Leptospirosis is a re-emerging zoonotic disease that can be transmitted to humans by direct or indirect contact with the contaminated urine of infected animals (Levett, 2001). Rodents are an important reservoir of *Leptospira* (Faine *et al.*, 1999; Levett, 2001). In Germany, leptospirosis is a notifiable disease with a low but increasing incidence (0.06 per 100 000 population, from 1998 to 2003) with 30–50 laboratory confirmed cases per year (Jansen *et al.*, 2005). The microagglutination test (MAT) is the standard method for serological diagnosis (Levett, 2001; WHO, 2003), but antibodies are not detectable in blood until 5 to 7 days after the onset of symptoms (Levett, 2003).

Since leptospire are difficult to culture, several PCR methods have been used to facilitate early diagnosis (Gravekamp *et al.* 1993; Bal *et al.*, 1994; Levett *et al.*, 2005;

Merien *et al.*, 2005). In this study, white fancy rats (domestic albino *Rattus norvegicus*) were identified as the potential infection source for acute leptospirosis in a human immunodeficiency virus (HIV)-positive patient by using a combination of bacteriological, serological, histological and molecular methods.

A 39-year-old male patient was hospitalised due to a fever of sudden onset, myalgia, arthralgia and icterus, and Weil's disease was suspected. The patient was immunosuppressed due to an HIV-infection (824 CD3 cells μl^{-1} , 250 CD4 cells μl^{-1} and 316 CD8 cells μl^{-1}). Histological examination of a needle biopsy of the liver showed canalicular cholestasis and ballooning of hepatocytes by haematoxylin and eosin staining, and spiral-like bacteria by Warthin–Starry silver staining. These results confirmed the putative diagnosis of leptospirosis and

helped to exclude HIV-related cholestasis (e.g. drug-induced hepatic injury or viral infection).

Blood samples taken 1 day after admission of the patient were analysed by *Leptospira*-specific PCR and bacterial culture. The sample gave positive results in a multiplex PCR with the G1/G2-B64I/II primers (Gravekamp *et al.*, 1993; Bal *et al.*, 1994) and classic PCR with the LipL32 F/B primers (Levett *et al.*, 2005), generating amplicons of 285 and 423 bp, respectively. However, bacterial culture (Faine *et al.*, 1999) was negative after 12 weeks, probably due to antibiotic treatment immediately after admission to the hospital. In a 9 month follow-up study, 6 sera were examined by MAT (Faine *et al.*, 1999; WHO, 2003) performed with *Leptospira* strains belonging to 10 serogroups and 15 serovars (Table 1). The first antibodies in serum were

Table 1. *Leptospira* reference strains and isolates

Species	Serogroup	Serovar	Strain/isolate	MAT*	MLVA1	MLVA2
<i>Leptospira interrogans</i>	Australis	Australis	Ballico	Yes	MLVA1-1	MLVA2-1
<i>Leptospira interrogans</i>	Australis	Bratislava	Jez Bratislava	Yes	MLVA1-2	MLVA2-2
<i>Leptospira interrogans</i>	Autumnalis	Autumnalis	Akiyami A	Yes	MLVA1-3	MLVA2-3
<i>Leptospira interrogans</i>	Bataviae	Bataviae	Swart	Yes	MLVA1-4	MLVA2-4
<i>Leptospira interrogans</i>	Canicola	Canicola	Hond Utrecht IV	Yes	MLVA1-5	MLVA2-5
<i>Leptospira interrogans</i>	Icterohaemorrhagiae	Copenhageni	M 20	Yes	MLVA1-10	MLVA2-11
<i>Leptospira interrogans</i>	Icterohaemorrhagiae	Icterohaemorrhagiae	Ictero no. 1	Yes	MLVA1-10	MLVA2-11
<i>Leptospira interrogans</i>	Pyrogenes	Pyrogenes	Salinem	Yes	MLVA1-8	MLVA2-10
<i>Leptospira interrogans</i>	Pomona	Pomona	Pomona	Yes	MLVA1-7	MLVA2-9
<i>Leptospira interrogans</i>	Sejroe	Hardjo	Hardjoprjitno	Yes	MLVA1-6	MLVA2-6
<i>Leptospira interrogans</i>	Sejroe	Saxkoebing	Mus 24	Yes	MLVA1-0	MLVA2-8
<i>Leptospira borgpetersenii</i>	Sejroe	Sejroe	M 84	Yes	MLVA1-0	MLVA2-0
<i>Leptospira borgpetersenii</i>	Sejroe	Istrica	Bratislava	No	MLVA1-0	MLVA2-0
<i>Leptospira borgpetersenii</i>	Sejroe	Hardjo (Hardjobovis)	Cattle isolate	No	MLVA1-0	MLVA2-7
<i>Leptospira borgpetersenii</i>	Tarassovi	Tarassovi	Perepelitsin	Yes	MLVA1-0	MLVA2-0
<i>Leptospira borgpetersenii</i>	Ballum	Ballum	Mus 127	Yes	MLVA1-0	MLVA2-0
<i>Leptospira borgpetersenii</i>	Javanica	Javanica	Veldrat Bataviae 46	No	MLVA1-0	MLVA2-0
<i>Leptospira kirschneri</i>	Grippotyphosa	Grippotyphosa	Moskva V	Yes	MLVA1-9	MLVA2-0
<i>Leptospira kirschneri</i>	Pomona	Mozdok	Swine isolate	No	MLVA1-0	MLVA2-0

*The strains were used for serological analysis.

demonstrated 1 week after admission of the patient, and the highest titre (1 : 3200 serovar Icterohaemorrhagiae) was found at day 21. Cross-reactions with the serovars Copenhageni (1 : 200) and Sejroe (1 : 100) were detected, especially in the acute phase of the illness. Such cross-agglutinations with M84 Sejroe after serovar Icterohaemorrhagiae, Canicola or Pomona infections have been described by others as well (Brem *et al.*, 1995). These unspecific reactions disappear during infection, when antibodies against the real causative agent become more predominant (Levett, 2001, 2003). Consequently, the presumptive causal agent of the infection belonged to *Leptospira interrogans* serogroup Icterohaemorrhagiae, since only serovars Copenhageni and Icterohaemorrhagiae (both with titres of 1 : 400) reacted in the last samples taken 9 months after admission.

The PCR technique, was very useful for early detection of leptospirosis, giving a positive result prior to seroconversion as described by other authors (Merien *et al.*, 2005; Gravekamp *et al.*, 1993; Bal *et al.*, 1994). The HIV status of the patient did not influence the clinical course of the infection or the MAT antibody response, which were similar to those observed in non-immunosuppressed patients (Jones & Kim, 2001).

In order to identify the source of infection, four white domestic rats owned by the patient were euthanized, and blood and kidney samples were taken. One kidney of each rat was tested for *Leptospira* by PCR analysis and bacterial culture. All four kidneys proved to be positive by both PCR methods (amplicons of 285 and 423 bp). Although cultures are relatively insensitive and require several weeks of incubation, all kidney samples were positive for *Leptospira* after 5 days as well. For serotyping, the four isolates were subjected to microagglutination (Faine *et al.*, 1999) with 15 rabbit sera representing the reference strains (Table 1). Cross-reactions with serovars Canicola and Pyrogenes were observed, but the highest titres were found with the antisera for serovars Copenhageni (1 : 6400–1 : 25 600) and Icterohaemorrhagiae (1 : 25 600–1 : 51 200).

Several molecular methods (including PFGE, random amplified polymorphic DNA analysis and RFLP) have been used

for the typing of *Leptospira* isolates (Perolat *et al.*, 1990; Taylor *et al.*, 1991). For this study, two variants of multilocus variable analysis (MLVA), MLVA1 (Majed *et al.*, 2005) and MLVA2 (Slack *et al.*, 2005), were carried out. The four isolates yielded the same profiles by MLVA1 and MLVA2 as the *L. interrogans* serovars Icterohaemorrhagiae and Copenhageni reference strains (Fig. 1). Similar to PFGE (Taylor *et al.*, 1991), MLVA typing was not able to differentiate between *L. interrogans* serovars Icterohaemorrhagiae and Copenhageni.

Serological analysis with mAbs at the Royal Tropical Institute, Amsterdam, Holland (World Health Organization/Food and Agriculture Organization of the United Nations Collaborating Centre for Reference and Research on Leptospirosis) revealed that the agglutination profile of the isolates examined with a panel of six mAbs was consistent with that of serovar Icterohaemorrhagiae with a slight

difference that might represent an antigenic variant that has been observed in Europe during the last few years (M. Goris, personal communication).

Altogether, this study suggests that the infection was due to *L. interrogans* serogroup Icterohaemorrhagiae, serovars Icterohaemorrhagiae or Copenhageni isolates originating from the patient's rats. The patient had no other known potential exposure to *Leptospira*. Unfortunately, direct isolates from the patient were not available and thus an absolute epidemiological link could not be established. However, there was strong diagnostic and epidemiological evidence that the pet rats were the source of infection. As far as we know, a relation between domestic rats held as pets and a severe leptospirosis in a HIV-positive patient has not been described before. During the last few years, the habit of keeping rats as pets has become a new fashion, and thus this kind of exposure

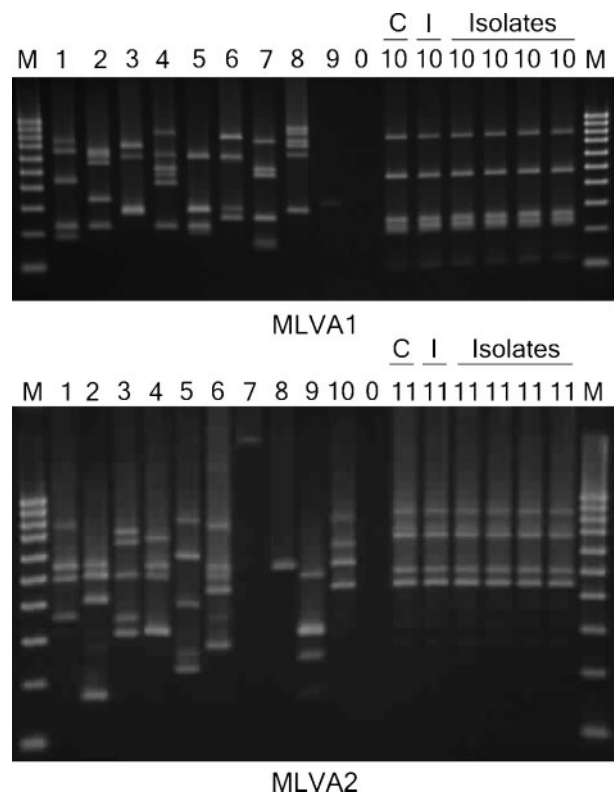


Fig. 1. MLVA typing of *Leptospira* strains. The band patterns shown are a mix of the PCR products obtained by analysis with each primer pair described by Majed *et al.* (2005) for MLVA1 and Slack *et al.* (2005) for MLVA2. C, Serovar Copenhageni reference strain; I, serovar Icterohaemorrhagiae reference strain; M, molecular marker HyperLadder IV (Bioline).

with infected rats may pose a threat for public health.

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