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Case Report

Severe course of rat bite-associated Weil's disease in a patient diagnosed with a new *Leptospira*-specific real-time quantitative LUX-PCR

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Leptospirosis is a zoonotic disease with global distribution, caused by spirochaetes of the genus *Leptospira*. Transmission of *Leptospira interrogans* serovar Icterohaemorrhagiae, the causative agent of Weil's disease, to humans usually results from exposure to the urine of infected, but mostly asymptomatic, rodents, either by direct contact or indirectly through contaminated soil or water. Although regarded as a re-emerging infectious disease, human leptospirosis is probably underdiagnosed due to its often unspecific clinical appearance and difficulties in culturing leptospires. Therefore, more rapid and specific diagnostic procedures are needed. Here we describe a novel real-time quantitative PCR system developed for the accurate and fast diagnosis of pathogenic *Leptospira* spp. Its usefulness in the management of a patient with rat bite-associated multiorgan failure is demonstrated.

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Introduction

Leptospirosis is a zoonotic disease with a global distribution, caused by spirochaetes of the genus *Leptospira*. It has recently been classified as a re-emerging infectious disease. Human infections are endemic in most tropical and moderate climates. Based on antigenic relatedness, more than 200 human pathogenic *Leptospira* serovars, mostly belonging to the species *Leptospira interrogans*, can be differentiated (Bharti *et al.*, 2003). A variety of both wild and domestic animals form the natural reservoir for pathogenic leptospires. Some serovars are associated with specific hosts, e.g. *L. interrogans* serovar Icterohaemorrhagiae, the causative agent of Weil's disease, which is primarily found in rats. Transmission to humans results from exposure to the urine of infected, but mostly asymptomatic, animals, either by direct contact or through contaminated soil or water. In central Europe the incidence of leptospirosis shows a seasonal pattern, with most infections being acquired from July to November (Jansen *et al.*, 2005; Baranton & Postic, 2006; Holk *et al.*, 2000). Although relatively rare in Germany, there are about 60 human cases per year (Jansen *et al.*, 2005), low but persisting rates of autochthonous leptospirosis exist. Due to its often unspecific clinical appearance, a lack of awareness among physicians, difficulties in isolating leptospires and the requirement to cultivate a broad range of *Leptospira* serovars for maintaining an adequate serological test system, leptospirosis is likely to be underdiagnosed. Moreover, due to long incubation times and the need to subcultivate diagnostic material, a rapid and specific diagnosis is often hard to achieve. In the following report, we describe a novel real-time quantitative PCR (qPCR) system based on Light Upon eXtension (LUX) technology and developed for the accurate and fast diagnosis of pathogenic *Leptospira* spp. Furthermore, we show its usefulness in the management of a patient with rat bite-associated multiorgan failure.

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Abbreviations: C_t, threshold cycle; FAM, 6-carboxyfluorescein; LUX, Light Upon eXtension; MAT, microscopic agglutination test; qPCR, quantitative PCR; T_m, melting temperature.

Methods

Reference strains and culture conditions. All reference strains (Table 1), representing the main pathogenic serovars of *Leptospira* spp. found in Germany, were cultured at 29 °C under aerobic conditions in solid Difco *Leptospira* medium base EMJH (Ellinghausen McCullough Johnson Harris; BD Bioscience) enriched with 10 % BSA, Tween 80/40. The cultures were maintained by weekly

Table 1. *Leptospira* reference strains used in this study

Serogroup	Serovar	Strain	Species
Australis	Australis	Ballico	*Leptospira interrogans*
Australis	Bratislava	Jez Bratislava	*Leptospira interrogans*
Autumnalis	Autumnalis	Akiyami A	*Leptospira interrogans*
Ballum	Ballum	Mus 127	*Leptospira borgpetersenii*
Bataviae	Bataviae	Swart	*Leptospira interrogans*
Canicola	Canicola	Hond Utrecht IV	*Leptospira interrogans*
Grippotyphosa	Grippotyphosa	Moskva V	*Leptospira kirschneri*
Hebdomadis	Hebdomadis	Hebdomadis	*Leptospira interrogans*
Icterohaemorrhagiae	Copenhageni	M 20	*Leptospira interrogans*
Icterohaemorrhagiae	Icterohaemorrhagiae	Ictero I	*Leptospira interrogans*
Javanica	Javanica	Veldrat Batavia 46	*Leptospira borgpetersenii*
Pomona	Pomona	Pomona	*Leptospira interrogans*
Pyrogenes	Pyrogenes	Salinem	*Leptospira interrogans*
Sejroe	Hardjo	Hardjoprajitno	*Leptospira interrogans*
Sejroe	Sejroe	M84	*Leptospira borgpetersenii*
Sejroe	Saxkoebing	Mus 24	*Leptospira interrogans*
Tarassovi	Tarassovi	Perepelitsin	*Leptospira borgpetersenii*

subculture into fresh medium. Cultures used were grown to stationary phase in 4–14 days, reaching a density of approximately 2×10^8 cells ml^{-1}.

Microscopic agglutination test (MAT). A MAT was carried out in microtitre plates using the 17 reference strains of pathogenic *Leptospira* as antigens. Serum samples and antigen were diluted (1 : 50) in PBS (pH 7.2). The plates were incubated at 29 °C for 1 h and then examined by darkfield microscopy for a first screening. Serial twofold dilutions (1 : 12.5–1 : 1600) were made of the positive serum samples that agglutinated leptospires in a 1 : 50 dilution and were screened against the reactive cultures in a second test run. The end point of the MAT reaction was determined as the dilution of sample that showed 50 % agglutination leaving 50 % non-agglutinated leptospires, as observed under darkfield microscopy (WHO & ILS, 2003).

Cultivation and identification of clinical isolates. Immediately at the time of sample collection from the rats, a *Leptospira* culture was initiated. A total of 8 ml semi-solid EMJH broth containing 10 % BSA, Tween 80/40 enrichment and 100 μg 5-fluorouracil ml^{-1} was inoculated with each of sterile kidney samples. The media were then transported at room temperature to the laboratory. A total of 0.5 ml was added to three different preparations of 6 ml semisolid EMJH media containing BSA, Tween 80/40 enrichment, 1 % rabbit serum, 0.1 % lactalbumin hydrosylate, which was further supplemented by (a) 10 μg 5-fluorouracil ml^{-1} and 10 μg vancomycin ml^{-1}, (b) 100 μg 5-fluorouracil ml^{-1} or (c) no antibiotics. The media were incubated at 29 °C for 6 months and examined periodically by darkfield microscopy. Positive cultures were further characterized as a *Leptospira* serovar by their reactivity with rabbit hyperimmune antisera (provided by the Koninklijk Instituut voor de Tropen, Royal Tropical Institute, Amsterdam, Netherlands) that had been raised against the 17 reference strains of pathogenic *Leptospira* (Table 1). A subsequent MAT was carried out using standard methods (WHO & ILS, 2003).

DNA preparation. Sample pre-preparation was performed by the following method: a small piece of kidney sample was transferred in a 2 ml plastic tube and frozen at −20 °C. Genomic DNA was extracted, after thawing of the samples, using the DNeasy blood & tissue kit (Qiagen), following the manufacturers' instructions. DNA samples of the kidney pieces were eluted from the kit columns to give a final volume of 200 μl. All DNA extraction experiments included a positive control consisting of 10 μl actively growing *Leptospira borgpetersenii* serovar Ballum strain Mus 127 culture and a negative control containing only the extraction buffers.

Primer design. Primers (Table 2) for conventional PCR were developed based on alignments of available *Leptospira* genomic DNA sequences obtained from the GenBank nucleotide sequence database at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) using Primer 3 (http://primer3.sourceforge.net/) to amplify a 433 or 344 bp fragment of the *lipL32* gene on chromosome I and a 515 bp fragment of the adenylate cyclase (Acl)-encoding gene on chromosome II, respectively. Primers for qPCR were created using D-LUX designer software (http://www.invitrogen.com/). The specificity of the primers in identifying only leptospiral sequences was assessed using the Basic Local Alignment Search Tool (BLAST) program and screening the NCBI sequence database. The primers for conventional PCR were synthesized by Thermo Electron and Operon Biotechnologies, and the fluorescence labelled primers were provided by Invitrogen.

qPCR. PCR amplification was performed in a total volume of 50 μl using 1× Platinum qPCR SuperMix-UDG (Invitrogen), 3 mM $MgCl_2$ (Invitrogen), 80 nM each LipL32 Primer [LipL32-287 FAM (6-carboxyfluorescein) labelled and LipL32-678] and 40 nM each Acl primer [Acl1 JOE (6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein) labelled and Acl3], 50 nM ROX reference dye (Invitrogen), 1fg plasmid 515 DNA (inhibition control), 5 μl sample DNA extraction and DEPC-treated H_2O (Invitrogen) to make up the final volume. Amplification and fluorescence detection were conducted in a MX3005P Stratagene instrument (software version 3.20/2006) using the following program in the SYBR green prototype experiment (dissociation curve): initial denaturation at 95 °C for 2 min, and 50 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min. The fluorescence reading was noted at the end of each annealing step. Finally, melting curve analysis was performed as follows: the PCR products were denatured at 95 °C for 2 min and then cooled to 55 °C for 30 s to allow annealing. The chamber temperature was then increased in 1 °C increments, with fluorescence measurements being

Table 2. Primers for PCR amplification of *Leptospira* spp. *lipL*32 gene and *acl* gene

Designation	5′→3′ sequence†	Length (bp)	PCR fragment length (bp)	Mol% G+C	T_m (°C)	Function
LipL32-287 FAM	CGAACTTAATCGCTGAAATGGGAGTTC*G	28	433	46.43	70.62	Forward LUX primer
LipL32-287	TAATCGCTGAAATGGGAGTTCG	22	433	45.45	63.45	Forward primer
LipL32-678	AGCAGACCAACAGATGCAACG	21	433	52.38	63.67	Reverse primer
LipL32-355	GCGGCWACCCCRGAAGAAAAAT	22	344	50.00	64.70	Forward semi-nested primer
Acl1 JOE	CGCAGTAACACCTCTCGTAATCTGC*G	26	515	53.85	68.80	Reverse LUX primer
Acl1	TAACACCTCTCGTAATCTGCG	21	515	47.62	58.07	Reverse primer
Acl3	CGTGGAAGTGGATGAGCACTGT	22	515	54.55	64.80	Forward primer

†International Union of Biochemistry codes: W, coding for T and A; R, coding for G and A. The loop structure of the LUX primer is underlined; C*, position for LUX dye labelling.

taken at every step. The melting temperature (T_m) was calculated automatically by the Stratagene software by plotting the first derivative of the raw fluorescence reading multiplied by −1 (-R'(T)) against temperature (°C) to determine the T_m of the sample.

qPCR controls. Each qPCR run included a positive extraction control, a negative extraction control and finally a negative PCR control, containing 5 µl DEPC-treated H_2O instead of DNA extract, to help in the detection of any possible presence of contaminating DNA in the test. Samples and controls were run in triplicate.

DNA standard. A control DNA standard was manufactured to test the sensitivity of the PCR and to quantify the leptospires in the clinical samples. A 433 bp long part of the *lipL32* sequence was amplified from *L. borgpetersenii* serovar Tarassovi strain Perepelitsin DNA using the primers LipL32-287 and LipL32-678. The PCR product was ligated into a pCR2.1-TOPO plasmid (Invitrogen) and then transformed into One Shot TOP10 chemically competent *Escherichia coli* cells (Invitrogen) and selected using blue/white screening as indicated in the TOPO cloning kit instructions. Luria–Bertani broth or agar supplemented with 50 µg ampicillin ml^{-1} was used for the culture of *E. coli*. Plasmid DNA was extracted from positive (white) colonies using the PureLink quick plasmid miniprep kit (Invitrogen), and the DNA concentration was measured using the BioPhotometer (Eppendorf) instrument. Plasmid DNA was adjusted to a concentration of 1 ng DNA μl^{-1} ($\approx 2 \times 10^8$ copies μl^{-1}) and 10-fold serial dilutions were performed up to 1 ag DNA μl^{-1} using TE buffer (pH 8.0) as the diluent. For sensitivity analysis a standard curve was created whereby each qPCR run included the FAM standard, ranging from 1×10^7 to 1×10^2 copies per reaction, in duplicate (Fig. 1). Sensitivity of the LUX-based qPCR was 1–10 genome copies per reaction.

Detection of PCR inhibitors. Each individual qPCR reaction tube contained a standard amount of plasmid 515 DNA (1 fg) corresponding to a known threshold cycle (C_t) value, between cycles 38 and 42 (threshold 0.0100), for detecting the presence of PCR inhibitors (inhibition control). A 515 bp long fragment of the *acl* gene on chromosome II was amplified from *L. interrogans* serovar Hardjo strain Hardjoprajitno DNA using the primers Acl3 and Acl1. The plasmid DNA preparation of positive bacteria was performed as described above. Inhibition control plasmid DNA was adjusted to a concentration of 1 ng DNA μl^{-1} ($\approx 2 \times 10^8$ copies μl^{-1}) and 10-fold serial dilutions in TE buffer (pH 8.0) were prepared to contain a minimum of 1 fg DNA μl^{-1}.

qPCR performance. The qPCR performance was assessed by analysing the specificity and efficacy of the PCR, as well as the reproducibility of replicates in different PCR runs.

Semi-nested PCR. Semi-nested amplification of the *lipL32* gene was performed in a total volume of 50 µl containing 1 µl qPCR products as the template, 1 × PCR buffer (Invitrogen), 1.5 units *Taq* DNA polymerase (Invitrogen), 0.2 mM each dNTP (Invitrogen), 1.5 mM $MgCl_2$ (Invitrogen), 100 nM each primer (LipL32-355 and LipL32-678) and DEPC-treated H_2O (Invitrogen) to make up the final volume. Thermal cycling was performed in a Biometra T3000 thermocycler as follows: initial denaturation at 95 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 65 °C for 40 s and 72 °C for 50 s, with a final extension step at 72 °C for 5 min. A total of 10 µl PCR product were subjected to electrophoresis in 3 % agarose gels run in 1× Tris/acetate EDTA (TAE) buffer pH 8.3 (Invitrogen) at 120 V for 45 min. The gel was visualized after staining with 0.5 µg ethidium bromide ml^{-1} (Sigma) and photographed using the BioDocAnalyse liveH system (Biometra). The sizes of the PCR amplified DNA fragments were estimated by direct comparison to a 50 bp ladder (Invitrogen) in each gel run.

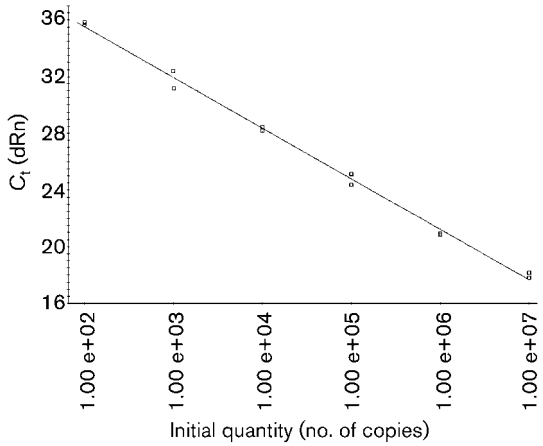

Fig. 1. Standard curve derived from the amplification plot of tenfold dilutions of *Leptospira* cloned DNA fragment (433 bp) used for quantification from 1×10^7 to 1×10^2 copies per reaction for the qPCR assay. Each dilution gives a C_t value (threshold 0.0100) that can be plotted against the number of copies to obtain a standard curve. The resulting slope demonstrates PCR efficiency. □, FAM standards ($r^2 = 0.996$); black line, FAM [$y = -3.572 \times \log(x) + 42.66$, efficiency = 90.5%.

Semi-nested PCR controls. Each semi-nested PCR run included a triplicate of no template control, the triplicates of qPCR product templates from the negative extraction controls and a negative PCR control, as well as a positive control (in duplicate), containing 1 µl low C_t copy number positive qPCR products.

Sequencing. Sufficient amounts of PCR products were directly sequenced by MWG Biotech using the original PCR primers: LipL32-287, LipL32-678, Acl1 and Acl3. Obtained sequences were aligned and compared with relevant sequences from the database using DNAMAN software (Lynnon Biosoft; version 5.2.9).

Case report

A 21-year-old immunocompetent woman fell ill with acute undifferentiated fever while visiting a friend. On the day before she had been bitten by her friend's tame pet rat. The rat belonged to a group of several rats that had been bought by the friend about 2 months previously from a pet shop. Two days later she developed intense pain in both calves, which was accompanied by vomiting and nausea on the next day. On hospital admission 1 day later, she had icterus and urinary excretion had ceased. Fever of 40 °C was persistent. The laboratory investigation showed elevated bilirubin (8.5 mg dl^{-1}), creatinine (3.8 mg dl^{-1}), urea (96 mg dl^{-1}) and slightly elevated transaminase levels. Furthermore, thrombocyte counts were low with 30 000 thrombocytes μl^{-1}. The patient was awake, did not show any signs of meningitis, but was hypotensive and had tachycardia. Echocardiography showed a diffuse hypokinetic myocardium. Chest X-rays suggested diffuse pulmonary oedema and infiltration. Under the suspected diagnosis of septic shock with multiple organ failure, a central venous and a dialysis catheter were inserted, and shock therapy with cristaloids and noradrenaline was initiated instantly after the patient has been transferred to the intensive care unit. Because of progressive respiratory insufficiency and haemoptysis, tracheal intubation and pressure-controlled ventilation was immediately necessary. Additionally, continuous haemofiltration was started to treat acute renal failure.

Due to haemorrhagic diathesis with pulmonary bleeding and haematuria, as well as her history of rat contact, a hantavirus infection was suspected. Leptospirosis, rat-bite fever due to *Streptobacillus moniliformis* (Elliott, 2007) or a fulminant sepsis caused by bacteria from the rat's oral flora were also included in the differential diagnosis list. However, all microbiological (e.g. blood cultures) and serological examinations (e.g. for hantavirus or *Leptospira* spp.) were negative. A second serological examination for hantavirus infection about 5 days later was also negative.

Since five pet rats – four young rats (nos 1–3 and 5) and the biting mother rat (no. 4) – still lived in the home of the patient's friend, they were seized by the local health authority for safety reasons under a presumed threat to public health, and for further microbiological diagnosis. Additionally, 35 littermates (nos 6–40) of the five rats were put under quarantine in the pet shop until a hantavirus infection could be excluded. Moreover, two pet rats (nos 41 and 42) – one of them also bought from the same pet shop at the same time as the five rats of the patient's friend– were seized in the patient's house about 500 km away from the friend's home.

The five rats of the patient's friend were clinically healthy and subsequently euthanized for pathological and microbiological examination in accordance with legal requirements. They showed no macropathological changes upon autopsy. From all five animals lung and kidney material, urine, heart blood and swabs of the oral cavity were obtained for microbiological and serological diagnosis. Molecular and serological diagnosis for Hantavirus infection performed at the Institute of Microbiology of the Bundeswehr was negative in all organ samples. Microbiological culture performed at the Bavarian Health and Food Safety Authority yielded no growth of *S. moniliformis* or other pathogenic bacteria. In contrast, leptospira DNA was detected by a novel qPCR (see Methods) from the kidney of the biting mother rat (no. 4). The DNA yield from a rice-grain sized kidney fragment corresponds to 73 750 leptospires. The *Leptospira*-qPCR on material of the four young rats as well as MATs for *Leptospira* antibodies on all five rats yielded negative results.

At the same time, while the patient's health continuously deteriorated under carbapenem therapy – given under the assumption of sepsis of unknown origin – with life-threatening cardial and pulmonal problems, a direct agglutination test (Bio-Rad) on the patient's serum obtained 7 days after her hospital admission showed antibodies against *Leptospira* spp. This patient serum, as well as a serum sample obtained 7 days previously, were re-examined by both a more sensitive MAT and an ELISA (Brem *et al.*, 1999), and showed a significant titre increase for *L. interrogans* serovar Icterohaemorrhagiae antibodies from 1 : 100 (intermediate) to 1 : 800 (strongly positive). During the following weeks the patient continuously improved under penicillin therapy and was discharged home. Penicillin was chosen since it is still considered to be the standard antimicrobial agent for the treatment of moderate-to-severe leptospirosis, although a recent prospective clinical study identified ceftriaxone as similarly effective (Panaphut *et al.*, 2003).

Later on, leptospires were grown from bacterial cultures of both the kidney material and the urine of the PCR-positive biting mother rat and characterized as *L. interrogans* serovar Icterohaemorrhagiae using rabbit hyperimmune antisera (titre 1 : 51 200; titres for serovar Copenhageni, Canicola and Hardjo were 1 : 3200, 1 : 400 and 1 : 200, respectively; titres for all other serovars were negative).

Discussion

The LUX-based qPCR presented here was developed for several reasons: (i) LUX-based technology offers a very cheap qPCR approach, since no probe is needed and one

primer pair – only one of the primers being labelled – is sufficient; (ii) in contrast to other real-time PCR methods, LUX-based qPCR runs on any real-time instrument platform; (iii) in contrast to previous 16S rDNA-based qPCR methods (Segura *et al.*, 2005; Smythe *et al.*, 2002; Viriyakasol *et al.*, 2006) detecting both pathogenic and apathogenic leptospires, our qPCR targets the *lipL32* gene present only in pathogenic *Leptospira* spp. and encoding the major outer membrane lipoprotein LipL32, a putative virulence marker of pathogenic *Leptospira* spp.; (iv) by using *acl* as an internal inhibition control no further specificity control via melting curve analysis has to be performed, in contrast another *lipL32*-based qPCR using SYBR green chemistry (Levett *et al.*, 2005) has to rely on melting point analysis for specificity reasons; (v) the sensitivity of our method is comparable to other qPCR methods detecting 1 to 10 genome copies per reaction.

To the best of our knowledge, only three clinical case reports on leptospirosis after a rat bite have been published (Cerny *et al.*, 1992; Gollop *et al.*, 1993; Luzzi *et al.*, 1987). The association of our patient's leptospirosis with a rat bite raises several clinical and epidemiological considerations. The leptospiral infection of the pet rat might have been due to the fact that the animal was repeatedly allowed to walk in its owner's garden where it might have acquired leptospires via the urine of wild-living mice or rats. The usual incubation period for leptospirosis in humans is about 1–2 weeks (Jauréguiberry *et al.*, 2005). In most cases human disease is transmitted by direct or indirect contact with urine, blood or tissue of asymptomatically infected rodents, mostly via abraded skin, but also may be transmitted via conjunctivae or the intact mucosal membranes in the oral cavity or the pharynx. Another important risk for infection is exposure to water contaminated with rodent urine, e.g. during leisure activities, such as water sports (Bharti *et al.*, 2003). Interestingly, in the three previously published case reports on rat bite-associated human leptospirosis the incubation period – 7 to 10 days – was considerably longer than in our patient (Cerny *et al.*, 1992; Gollop *et al.*, 1993; Luzzi *et al.*, 1987).

When considering the rat bite as the cause of the patient's infection, the unusually acute onset of leptospirosis within a few hours after the bite could be explained by the fact that the rat's infection itself might also have been very acute. Under these circumstances, leptospires might have been carried by the rodent's saliva within a short time of generalization, and during this time they were transmitted to the patient by the bite (Hanson, 1982). An indirect finding supporting this hypothesis is that both the 4 younger rats and the 35 littermates from the pet shop were negative for leptospirosis both in MAT serology and in qPCR analysis; therefore, no possibility of transmission from the biting rat to the other rats was given, possibly indicating that the infection in the biting rat had happened very recently. A further – albeit indirect – clue is the fact that the leptospiral load in the rice grain-sized kidney tissue fragment of rats no. 41 and no. 42 seized in the patient's house were significantly higher (330 800 and 788 000 leptospires, respectively) than in that of rat no. 4 (73 750 leptospires) according to the respective qPCR results. Although quantification of leptospires by our LUX-based qPCR is very reliable, as tested both in plasmid titration assays (see Methods), and in a large veterinary clinical study involving 126 vitreous and aqueous humour samples from horses suffering from leptospiral equine recurrent uveitis, as well as 379 samples from healthy control horses (A. Roczek, unpublished data), the significance of the leptospiral burden in the three rats has to be interpreted with caution, since to the best of our knowledge no data regarding the leptospiral load in naturally infected rodents are available.

Arguments against the hypothesis of an acute and generalized infection in the biting rat (no. 4) allowing saliva-dependent transmission to our patient are: (i) the unaffected health status of the biting rat, including its normal pathological findings, and (ii) the fact that the patient's friend had been repeatedly bitten by the same rat and did not develop overt disease.

A more probable explanation for the short time between bite and symptoms in our patient would be that leptospires from the rat's urine – maybe sprinkled on the patient's skin in a considerable quantity while urinating in a panic attack – were injected into the patient by the rodent's teeth. The resultant high bacterial inoculation load might be responsible for the fulminant and life-threatening onset of Weil's disease in our patient.

Alternatively, our patient might have been infected earlier by the 'classical' route via direct or indirect contact with rat urine and therefore developed her symptoms after a longer incubation time. This possibility is supported by the fact that the two rats (no. 41 and no. 42) seized in her own house were also *Leptospira* DNA positive in their kidney tissues when tested by qPCR. Interestingly, sequencing of both the *acl* (515 bp) and the *lipL32* (433 bp) genes of all three rat PCR products (from rats 4, 41 and 42) revealed 100 % sequence identity, suggesting a common strain infecting the three rodents. Since rat 41 and biting rat 4 were purchased from the same pet shop, these sequence identities might indicate that both rats had been infected already in the pet shop. However, neither the rats kept with rat no. 4 (i.e. rats 1–3 and 5) nor the 35 pet shop littermates (rats 6–40) were qPCR-positive. Another common infection source for rats 4, 41 and 42 could not be elucidated.

Considering a classical infection route in our patient, with an usual incubation period of 1–2 weeks, the bite association would only be coincidental with the beginning of her symptoms. Even additional, and possibly more sophisticated, typing methods would not be able to definitively disclose the route of transmission in our patient, since the patient's leptospirosis was only diagnosed serologically and no *Leptospira* DNA had been isolated from patient material.

Taken together, this case illustrates the usefulness of a rapid and specific *Leptospira* qPCR method, as well as a

multidisciplinary approach involving both medical and veterinary expertise, for the management of a patient with a haemorrhagic fever-like clinical presentation.

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