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SHORT COMMUNICATION

# Occurrence of *Leptospira* DNA in water and soil samples collected in eastern Poland

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## Abstract

*Leptospira* is an important re-emerging zoonotic human pathogen, disseminated by sick and carrier animals, water and soil. Weather calamities, such as flooding or cyclones favour the spreading of these bacteria. To check a potential role of natural water and soil in the persistence and spread of *Leptospira* on the territory of eastern Poland, 40 samples of natural water and 40 samples of soil were collected from areas exposed to flooding, and 64 samples of natural water and 68 samples of soil were collected from areas not exposed to flooding. Samples of water were taken from various reservoirs (rivers, natural lakes, artificial lakes, canals, ponds, farm wells) and samples of soils were taken at the distance of 1–3 meters from the edge of the reservoirs. The samples were examined for the presence of *Leptospira* DNA by nested-PCR. Two out of 40 samples of water (5.0%) collected from the area exposed to flooding showed the presence of *Leptospira* DNA, while all 40 samples of soil from this area were negative. All samples of water and soil (64 and 68, respectively) collected from the areas not exposed to flooding were negative. No significant difference were found between the results obtained in the areas exposed and not exposed to flooding. In conclusion, these results suggest that water and soil have only limited significance in the persistence and dissemination of *Leptospira* in eastern Poland.

## Key words

*Leptospira*, water, soil, epidemiology, eastern Poland

## INTRODUCTION

Leptospirosis is an infectious disease caused by spirochetes belonging to the genus *Leptospira*, comprising both pathogenic and saprophytic species. It is regarded as the most widespread zoonosis, which is re-emerging worldwide as a major public health problem. Humans can become infected directly through contact with animals, such as rodents and domestic animals (dogs, cattle and swine), or indirectly through water and soil contaminated with the urine of infected animals [1, 2, 3, 4, 5, 6, 7, 8]. Weather calamities, such as heavy rainfall, flooding, cyclones or typhoons favour the spreading of these bacteria [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19].

To check the potential role of water and soil in the persistance and spread of *Leptospira* on the territory of eastern Poland, samples of natural water and soil were collected from areas exposed and not exposed to flooding, and subsequently examined for the presence of *Leptospira* DNA by PCR.

## MATERIALS AND METHOD

**Collection of water and soil samples.** A total of 104 samples of natural water and 108 samples of soil were collected on the territory of Lublin province (eastern Poland). Of these, 40 samples of water and 40 samples of soil were collected in the years 2010–2011 in an area of the north-western part of province exposed to flooding from the river Vistula, while the remaining 64 samples of water and 68 samples of soil were collected in the years 2012–2013 in the areas not exposed to flooding, comprising the suburban area near the city of Lublin and the area of thee Łęczyńsko-Włodawskie Lakeland. Out of 104 samples of natural water, 39 samples were taken from streams, 22 from natural lakes, 6 from artificial lakes, 17 from ponds, 6 from canals, and 14 from farm wells. Out of 108 samples of soil, 104 were taken near the sampled sources of water at a distance of 1–3 meters from the edge, and 4 were taken in crop fields in the suburban area not exposed to flooding.

Samples of water were taken into 5 L sterile plastic containers and kept at room temperature until examination, a period not exceeding 3 days. Samples of soil were taken with a sterile spatula into Falcon™ 50 mL sterile plastic tubes and kept at the temperature -20 °C until examination.

**DNA isolation.** Water samples were filtered first through cellulose Millipore filters with a pore size of 0.45 µm, and then through cellulose Millipore filters of the pore size 0.22 µm (Sterile S-PAK Membrane Filters, Millipore Corporation, Billerica, MA, USA). The sediment was scraped off the filters and suspended in PBS in Eppendorf® tubes. Next, a series of centrifugations was performed, each at 5,000 × g for 10 min. The supernatant was removed and the remaining sediment suspended in 20–30 µl PBS for DNA isolation. Bacterial DNA was isolated from the sediment with the commercial Qiamp DNA Mini Kit (Qiagen, USA), according to manufacturer's instruction, and following the protocol for DNA isolation from Gram-negative bacteria.

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Soil samples weighing 200–250 mg were used for isolation of bacterial DNA with the commercial DNA Stool Mini Kit (Qiagen, USA), according to manufacturer's instructions.

The amounts and purity of the isolated nucleic acids were measured with a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific, USA).

**PCR assay.** The semi-nested PCR method was used to identify DNA of *Leptospira* spp. in the water and soil samples.

***Leptospira* spp. DNA identification.** A gene fragment of LipL32 lipoprotein was used as a genetic marker to detect *Leptospira* spp. DNA [20]. Identification was carried out using a pair of primers (Eurogentec, Seraing, Belgium): Amu1 (5'- CGC GCT GCA GTT ACT TAG TCG CGT CAG AAG-3') and Amu2 (5'- CGC GGT CGA CGC TTT CGG TGG TCT GCCAAG c-3') for amplification of the fragment of LipL32 gene. For semi-nested PCR reaction, the primers Amu2 and AmuN (5'-CTA TGT TTG GAT TCC TGC-3') were used. First, PCR reaction in a final volume of 25 µl contained: 0.625 U (0.125 µl) of *Taq* DNA polymerase (Qiagen, USA), 1′ PCR buffer (2.5 µl) containing 15 mM MgCl2 (Qiagen, USA), 2.5 µl 2 mM dNTPs (final concentration 0.2 mM) (Fermentas, Vilnius, Lithuania), 1.25 µl of 10 µM of each Amu1 and Amu2 primers, 14.875 µl nuclease-free water (Applied Biosystems Inc., USA), and 2.5 µl of matrix DNA from water or soil isolates.

The reaction was performed in a C1000 Thermal Cycler (BioRad), and consisted of the initial denaturation (3 min at 94°C) and 35 cycles; each of them included the proper denaturation (30 sec at 94°C), primers annealing (30 sec at 55°C), elongation (60 sec at 72°C), and the final elongation (7 min at 72°C). Electrophoresis was performed in 2% agarose gels in standard conditions. The gels were stained with ethidium bromide and read under UV light. 756 bp-long electrophoresis strips were considered positive.

As the positive control, the thermally-inactivated suspensions of following strains were used: *Leptospira interrogans* serovar Icterohaemorrhagiae, *Leptospira kirschneri* serovar Grippotyphosa, *Leptospira borgpetersenii* serovar Tarassovi, and *Leptospira interrogans* serovar Pomona (strains obtained from the National Veterinary Research Institute, Puławy, Poland). The negative control, instead of matrix DNA, was nuclease-free water.

Amu2 and AmuN primers were used in re-amplification. 25 µl of the reaction mixture contained: 1.25 U (0.25 µl) of *Taq* DNA polymerase (Qiagen, USA), 1′ PCR buffer (2.5 µl) (Qiagen, USA), 1.25 µl of 2 mM dNTPs (final concentration 0.1 mM) (Fermentas, Vilnius, Lithuania), 1.25 µl of 10 µM of each primer, 16.0 µl nuclease-free water (Applied Biosystems Inc., USA), and 2.5 µl of matrix DNA from first PCR 10′ diluted in nuclease-free water.

The time temperature profile of the reaction was identical to the previous one, with the exception of the primers annealing which was performed at the temperature 58°C. Reaction products were detected in 2% agarose gels in the standard electrophoresis conditions. After ethidium bromide staining, the strips were read under UV light. The samples with a 574 bp-long strip were considered positive.

**Statistical analysis.** The data were analysed by Student's t-test with the use of STATISTICA for Windows v. 5.0 package (StatSoft Inc., Tulsa, Oklahoma, USA).

## RESULTS

Two out of 40 samples of water (5.0%) collected from the area exposed to flooding showed the presence of *Leptospira* DNA, while all 40 samples of soil from this area were negative (Tab. 1). The only 2 positive samples originated from the farm wells. All samples of water and soil (64 and 68, respectively) collected from the areas not exposed to flooding were negative (Tab. 2). No significant difference could be found between the results obtained on the areas exposed and not exposed to flooding (water samples P=0.0738; soil samples P=1.000). The occurrence of *Leptospira* DNA in total water samples was 1.9% and in total soil samples it was equal to zero.

**Table 1.** Occurrence of *Leptospira* DNA in samples of natural water and soil from areas exposed to flooding

| Source of the samples* | Water | Soil |
|---|---|---|
| | positive/examined (percent) | positive/examined (percent) |
| Streams | 0/17 (0) | 0/17 (0) |
| Ponds | 0/7 (0) | 0/7 (0) |
| Canals | 0/2 (0) | 0/2 (0) |
| Farm wells | 2/14 (14.3%) | 0/14 (0) |
| Total | 2/40 (5.0%) | 0/40 (0) |

*Samples of water were collected from the water reservoir and samples of soil collected at a distance of 1–3 meters from the edge of the reservoir.

**Table 2.** Occurrence of *Leptospira* DNA in samples of natural water and soil from areas not exposed to flooding.

| Source of the samples* | Water | Soil |
|---|---|---|
| | positive/examined (percent) | positive/examined (percent) |
| Streams | 0/22 (0) | 0/22 (0) |
| Natural lakes | 0/22 (0) | 0/22 (0) |
| Artificial lakes | 0/6 (0) | 0/6 (0) |
| Ponds | 0/10 (0) | 0/10 (0) |
| Canals | 0/4 (0) | 0/4 (0) |
| Crop fields | n. t. | 0/4 (0) |
| Total | 0/64 (0) | 0/68 (0) |

*Samples of water were collected from the water reservoir and samples of soil collected at a distance of 1–3 meters from the edge of the reservoir.
n. t. = not tested.

## DISCUSSION

The low percentage of water samples containing DNA of *Leptospira* noted in this study is comparable to the results of Vital-Brazil et al. [5] who found DNA of the mentioned spirochetes in 3% of water samples collected in an urban area in Brazil, and to the results reported from Chile [21], where 3.9% of water samples collected from rivers or irrigation channels showed in PCR positive results. The results of the presented study are also similar to those recorded by Karaseva et al. [22] in Russia, who isolated spirochetes of genus *Leptospira* from 1.1% of investigated soil samples, and to those obtained by-Yang et al. [23] in China, who isolated them from 2.14% of water samples and 4.9% of soil samples. The occurrence noted in the current study is lower compared to those obtained with PCR by French investigators [24] in waters associated with human leptospirosis cases (13.2%), but very similar to the results obtained by these authors in the control waters not associated with such cases (0.9%).

On the other hand, the percentage of positive results found in the presented study is distinctly lower compared to those obtained previously in the USA with the use of isolation by culture, where Tripathy and Hanson [25] noted a 64.3% prevalence of *Leptospira* in water samples, and Henry and Johnson [26] recorded a 42% prevalence of saprophytic leptospires in water samples and the 57% prevalence in soil samples. A high prevalence of *Leptospira* was found also by culture in water and soil samples collected in some Asian countries; Saito et al. [27] reported 42.5% and 40%, respectively, from the Philippines and Japan; Ridzlan et al. [28], 23.1% and 23.3%, respectively, from Malaysia, and Benacer et al. [29] 10.3% and 10.7%, respectively, also from Malaysia. Cann et al. [30] analyzing evidence from scientific literature confirm that most reported outbreaks of *Leptospira* spp. following extreme water-related weather events occurred in North America or Asia. Although in Europe the occurrence of pathogenic leptospires in the environment seems to be lower, authors from this continent [31, 32] noticed that in some countries over 50% of cases of leptospirosis were reported as environment- or water-related [21].

Some limitations of the presented study concern the character of the water reservoirs in the areas exposed and not exposed to flooding, which are not fully comparable (more farm wells were examined in the exposed area and more natural lakes in the unexposed area), and no differentiation of the *Leptospira* isolates into the pathogenic and saprophytic strains. Nevertheless, these limitations do not affect the final conclusion – a low content of *Leptospira* in the examined samples.

In conclusion, the results suggest that water and soil have only limited significance in the persistence and dissemination of *Leptospira* in eastern Poland.

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