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Leptospira Exposure in Urban River Surfers, Germany, 2024

Appendix

Materials and Methods

Study design and participants

We conducted a controlled cross-sectional study between September 4 and November 7, 2024, comparing Eisbach river surfers in Munich with non-surfing controls selected to achieve a similar age and sex distribution. No individual matching was performed. Surfers were recruited on site at the Eisbach river surfing wave in Munich (coordinates: 48°08'36.9"N, 11°35'16.1"E), an urban side arm of the Isar River. Controls were recruited among employees of TUM University Hospital after enrollment of surfers had begun. During control recruitment, age and sex distribution were monitored to achieve approximate comparability with the surfer group. Controls reported no river surfing exposure.

The eligibility criteria required surfers to have surfed the Eisbach River at least three times within the previous 12 months, whereas controls must not have surfed during this period. The exclusion criteria for both groups were age <18 years and a history of leptospirosis. No formal sample size calculation was performed. The study size was determined pragmatically by feasible on-site recruitment of Eisbach surfers during the study period and subsequent recruitment of a non-surfing control group with a similar age and sex distribution.

All participants provided written informed consent and completed a standardized questionnaire covering demographics, travel history, animal contact, and compatible symptoms within the preceding 12 months; an English translation of the questionnaire is provided below. They also provided blood samples for serological and polymerase chain reaction (PCR) testing. Serologic analyses included all participants with available blood samples (102 surfers and 100 controls). Questionnaire data were available for 100 surfers and 98 controls and were used for

baseline, exposure, and symptom characteristics. Countries were classified as having a high risk of leptospirosis according to the CDC Yellow Book (*1*).

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Technical University of Munich (approval number: 2024-434-S-SB). The study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Sampling procedures

Blood samples were obtained from participants upon enrollment between September 4 and November 7, 2024. Environmental samples were collected on five sampling days between September 4 and September 29, 2024. Environmental sampling sites were selected a priori to represent locations with plausible direct contact by surfers during routine surfing activity. Water and soil samples, including waterlogged soil and puddles, were collected on five sampling days from the river water at the Eisbach wave and from the surfers' entry and exit areas on both riverbanks, resulting in a total of 15 environmental samples. Samples were stored at 5 °C and regularly transported to the *Leptospira* Reference Laboratory of the German Federal Institute for Risk Assessment (BfR), Berlin, Germany for analysis.

Laboratory methods

Serological testing and PCR were performed at the BfR. Serum samples were screened for anti-leptospiral IgG and IgM antibodies using an in-house enzyme-linked immunosorbent assay (ELISA) (*2*). IgM results were expressed as percent optical density (OD%) relative to the assay cutoff and interpreted as follows: negative <40 OD%, borderline ≥40 and <56 OD%, and positive ≥56 OD%. IgG results were expressed as OD% and interpreted as follows: negative <86 OD%, borderline ≥86 and <98 OD%, and positive ≥98 OD%, according to internal laboratory criteria.

Samples that tested positive for IgG by ELISA were further analyzed using a microscopic agglutination test (MAT). MAT was performed using 17 reference strains: Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo, Hebdomadis, Pomona, Saxkoebing, Sejroe, Tarassovi, Ballum, Icterohaemorrhagiae, Pyrogenes, and Javanica. A MAT titer ≥1:100 was considered positive (*3*).

DNA was extracted from ethylenediaminetetraacetic acid blood using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), and from environmental samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions (*4*). PCR targeting *lipL32* was performed as previously described, with slight modifications (*5*). When possible, PCR-positive samples were subjected to additional typing using *secY* single-locus and multilocus sequence typing.

Indicators of regional leptospirosis activity

Surrogate indicators of leptospirosis activity and risk factors for leptospirosis transmission in the greater Munich region were analyzed: reported human leptospirosis cases, canine leptospirosis requiring hospital treatment, and rat infestation estimates.

Human leptospirosis notification data for Munich during 2016–2024 were obtained from the Munich Department of Health. In Germany, direct or indirect laboratory evidence of acute infection with human-pathogenic *Leptospira* spp. is notifiable under §7 of the Infection Protection Act. Notifications received by the Munich Department of Health were assessed according to national reporting criteria defined by the Robert Koch Institute and forwarded to the Bavarian Health and Food Safety Authority when these criteria were met. Because public health follow-up may determine that a notification does not represent a confirmed leptospirosis case, we analyzed these data as notification counts rather than confirmed case counts and used them only as contextual indicators of regional leptospirosis activity. Available public health and clinical documentation identified Isar River or Eisbach exposure in 12 notified persons, including 10 with documented Eisbach surfing. These included the 4 hospitalized surfers who prompted the present study; in 1 of these notifications, Isar River or Eisbach exposure was documented, but surfing was not explicitly captured in the notification record. Recreational water exposure was not systematically recorded throughout the full observation period. Contextual surveillance analyses were restricted to 2016–2024 because regular surfing exposure at the Eisbach wave was not comparable in 2025. Surfing activity was substantially disrupted by two exceptional events that had not occurred during the preceding observation years: a closure of more than two months after a fatal surfing accident and the disappearance of the wave after annual drainage and maintenance of the river channel from October 2025 onwards.

Rat infestation data for the Munich City area (2015–2024) were obtained from the Municipal Health Department. The infestation numbers were based on estimates of the on-site infestation density from officially confirmed infestation reports. No surveys were conducted in 2020 because of Coronavirus Disease 2019 restrictions.

Canine leptospirosis cases treated at the LMU Small Animal Clinic, Munich, were retrospectively identified using diagnostic codes and free-text searches in the electronic clinical documentation system and by review of dialysis records from October 2014 to December 2024. Identified cases were verified using individual clinical records.

Local climate data

Climate data were obtained in collaboration with the German Weather Service and from publicly available data provided by the Bavarian State Office for the Environment (LfU; https://www.gkd.bayern.de). The annual average water temperature of the Eisbach River (2015–2024) and the annual average ambient temperature in the Munich region (2000–2024) were analyzed. Annual averages were calculated as the mean of the monthly averages derived from daily measurements. Data were collected for each observation period.

The temporal overlap between inpatient leptospirosis cases at TUM University Hospital and periods of flooding on the Isar River in Munich was assessed descriptively. Flooding was defined as a water level ≥240 cm (alert level 1) according to the Bavarian Flood Warning Service (*6*)

Statistical analysis

Statistical analyses were performed using R (version 2025.09.1+401, Vienna, Austria). Temporal trends were visualized using LOESS smoothing with a span of 0.75 and tested for significance using a linear regression model. The slope coefficient was used to quantify the average annual change, and significance was assessed using a two-sided Wald test. For continuous variables, the Mann–Whitney U test was used for group comparisons. Categorical variables were assessed using the chi-squared test, or Fisher's exact test if the expected frequency under the null hypothesis was <5 in at least one cell of the cross table. For the primary serological comparison, group differences were analyzed using a one-sided Fisher's exact test, reflecting the a priori directional hypothesis that *Leptospira* IgG seroreactivity would be higher among surfers than among controls. Consequently, we used a one-sided test at $\alpha = 0.05$ and

report the corresponding two-sided 90% confidence interval, whose lower bound is equivalent to that of a one-sided 95% confidence bound. The corresponding two-sided Fisher exact p-value is provided for transparency. All other analyses were exploratory and used two-sided tests and two-sided 95% confidence intervals, where applicable. For each test, a significance level of 5% was used. Observations with missing values were excluded from the analysis.

Participant questionnaire

The questionnaire was administered using the LamaPoll online survey platform. The following is an English translation of the standardized questionnaire used for study participants.

Questionnaire for river surfers

- How old are you?
- What sex were you assigned at birth?
- What is your occupation?
- Do you have a known liver or kidney disease?
- Have you traveled abroad during the past 12 months? If yes, where?
- Do you have pets? If yes, which animals?
- For how many years have you been surfing at the Eisbach?
- How often do you surf at the Eisbach per month?
- During the past 12 months, have you had one or more of the following symptoms?
    - Fever
    - Conjunctivitis
    - Yellowing of the eyes
    - Muscle pain

- If yes, had you surfed at the Eisbach during the 14 days before symptom onset?

Questionnaire for controls

- How old are you?
- What sex were you assigned at birth?
- What is your occupation?
- Do you have a known liver or kidney disease?
- Have you traveled abroad during the past 12 months? If yes, where?
- Do you have pets? If yes, which animals?
- During the past 12 months, how often did you swim in rivers or lakes per month on average?
- In which body of water did you swim most frequently during this summer?
- During the past 12 months, have you had one or more of the following symptoms?
    - Fever
    - Conjunctivitis
    - Yellowing of the eyes
    - Muscle pain
- If yes, had you swum in a river or lake during the 14 days before symptom onset?

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Appendix Table 1. Self-reported symptoms within the past 12 months of surfers and controls*

	Surfers		Controls	
Variable	Seronegative	Seroreactive	Seronegative	Seroreactive
No symptoms	55 (61%)	6 (60%)	54 (57%)	2 (67%)
Fever	28 (31%)	4 (40%)	35 (37%)	1 (33%)
Eisbach surfing ≤ 14 d	19 (68%)	2 (50%)	-	-
Conjunctivitis	4 (4%)	1 (10%)	4 (4%)	0 (0%)
Eisbach surfing ≤ 14 d	4 (100%)	0 (0%)	-	-
Jaundice	1 (1%)	0 (0%)	1 (1%)	0 (0%)
Eisbach surfing ≤ 14 d	1 (100%)	-	-	-
Myalgia	14 (16%)	2 (20%)	6 (6%)	0 (0%)
Eisbach surfing ≤ 14 d	10 (71%)	1 (50%)	-	-

*Self-reported symptoms during the preceding 12 months, stratified by IgG seroreactivity. Seroreactivity was defined as an IgG-positive or borderline IgG ELISA result. Percentages for symptom rows refer to participants in the respective serostatus group; percentages for "Eisbach surfing ≤14 d" refer to participants reporting the corresponding symptom.

Appendix Table 2. Surfing exposure and risk factors among surfers*

Variable	Seronegative	Seroreactive	Effect measure	*p*-value
Years surfing at the Eisbach river (median, years)	4 (IQR 1–8.5)	4 (IQR: 2–10.5)	HL (95% CI): 1.00 (-2.00 to 4.00)	0.6083 (MWU)
Surf sessions per month (median)	10 (IQR: 4–12.75)	10 (IQR: 6–12)	HL (95% CI): -0.00 (-5.00 to 2.00)	0.6748 (MWU)
Recent travel abroad (N, %)	83 (92%)	8 (80%)	OR (95% CI): 0.34 (0.05 to 3.92)	0.2212 (F)
Travel to high-risk countries	31 (34%)	2 (20%)	OR (95% CI): 0.48 (0.05 to 2.61)	0.4900 (F)
Frequent animal contact	33 (37%)	3 (30%)	OR (95% CI): 0.74 (0.12 to 3.53)	>0.9999 (F)

*Surfing exposure and potential risk factors among Eisbach surfers, stratified by IgG serostatus. Continuous variables are presented as medians and interquartile ranges (IQR), and categorical variables are presented as absolute numbers and percentages. Effect measures compare seroreactive with seronegative surfers. Hodges–Lehmann (HL) estimates are reported for continuous variables and odds ratios (OR) for categorical variables. Comparisons between seronegative and seroreactive participants were conducted using the Mann–Whitney U test (MWU) for continuous variables and Fisher's exact test (F) for categorical variables.

Appendix Table 3. Sensitivity analysis excluding participants with travel to high-incidence countries*

Group	**Seroreactive**	**Seronegative**	**Seroreactivity**
Surfers without travel to high-incidence countries (N, %)	6	55	6/61 (9.8%)
Controls without travel to high-incidence countries (N, %)	3	71	3/74 (4.1%)

*Sensitivity analysis restricted to participants with available travel data and no travel to high-incidence countries during the preceding 12 months. Seroreactivity was defined as an IgG-positive or borderline IgG ELISA result. Percentages were calculated as the number of seroreactive participants divided by the total number of participants in each group.



Appendix Figure. Human leptospirosis notifications reported to public health authorities in Munich, 2016–2024. All notifications meeting national reporting criteria were included, irrespective of final confirmation status. Linear regression estimated an average annual increase of 1.35 (95% CI 0.01–2.69) notifications/year (p = 0.049 by 2-sided Wald test; R^2 = 0.45).