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A predictive risk map for human leptospirosis guiding further investigations in brown rats and surface water

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ABSTRACT

Leptospirosis is a zoonosis caused by the spirochete *Leptospira* spp. It is often not clear why certain areas appear to be hotspots for human leptospirosis. Therefore, a predictive risk map for the Netherlands was developed and assessed, based on a random forest model for human leptospirosis incidence levels with various environmental factors and rat density as variables. Next, it was tested whether misclassifications of the risk map could be explained by the prevalence of *Leptospira* spp. in brown rats. Three recreational areas were chosen, and rats (≥25/location) were tested for *Leptospira* spp. Concurrently, it was investigated whether *Leptospira* spp. prevalence in brown rats was associated with *Leptospira* DNA concentration in surface water, to explore the usability of this parameter in future studies. Approximately 1 L of surface water sample was collected from 10 sites and was tested for *Leptospira* spp. Although the model predicted the locations of patients relatively well, this study showed that the prevalence of *Leptospira* spp. infection in rats may be an explaining variable that could improve the predictive model performance. Surface water samples were all negative, even if they had been taken at sites with a high *Leptospira* spp. prevalence in rats.

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Introduction

The zoonosis leptospirosis is a re-emerging disease caused by the spirochete *Leptospira* spp [1]. It has a global spread and is estimated to cause 1.03 million human illness cases and 58,900 deaths each year, predominantly occurring in poorer, tropical regions [2]. A large number of mammalian hosts can harbor and excrete the pathogen from their renal tubules [3]. Brown rats (*Rattus norvegicus*) are considered a major source of *Leptospira* infection to humans through direct and indirect transmission. They are the main reservoir for *L. interrogans* serogroup Icterohemorrhagiae. Human infection with this serogroup may be asymptomatic or mild but can also cause disease ranging from acute febrile illness to hepato-renal disorders associated with bleeding tendency, which is referred to as Weil's disease or Weil's syndrome [4].

In the Netherlands, human leptospirosis has been a notifiable disease since 1928 [5]. About 9 to 17 cases per year were reported in the period 2010–2013, but in 2014, the number of reported autochthonous leptospirosis patients in the Netherlands increased fourfold [6]. This number only slowly decreased in the years thereafter but increased again in 2019 and 2020, and slightly decreased in 2021 [7] (personal communication R. Pijnacker).

Most of the autochthonous *Leptospira* spp. cases are related to recreation, e.g. swimming, or to occupational activities, mostly among farmers [6,8]. Areas with a high incidence of human leptospirosis can be found in central and northern parts of the Netherlands.

However, the drivers underlying this spatial pattern in incidence of leptospirosis remain unclear. A study of leptospirosis in rats showed that prevalence of leptospires ranged from 33% to 59% in four different areas in the Netherlands, while no difference was observed between areas with high or low human incidence [9]. Another study identified driving environmental variables associated with leptospirosis: coverage of arable land, built-up area, grassland, and sabulous clay soils were significant variables [10]. However, this model could not fully explain the spatial patterns that were seen by only using environmental risk factors on land-use, soil properties, etc. Therefore, it seemed promising to develop a model including rat densities.

To study the origin of the spatial differences that are seen in the leptospirosis distribution in the Netherlands, leptospirosis was modelled using various environmental factors, including rat population densities. The resulting model still had various areas for which the

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predicted risk differed from the actual risk, e.g. some areas had higher or lower numbers of patients than would be expected based on the model. To further investigate whether *Leptospira* spp. prevalence in rats could be an explanation for the misclassification, rats were captured at three study sites close to recreational water. Concurrently, water samples were collected at these sites, to assess the potential use of detection of *Leptospira* spp. in water as a fast and relatively inexpensive alternative for detection in rats.

Methods

Development of the predictive risk map

Data collection – Human cases: Cases were confirmed positive for *Leptospira* spp. as described in Pijnacker et al. [6]: 'when positive by culture and/or PCR and/or serology (MAT or IgM ELISA) and with fever or at least two of the following symptoms: rigors, headache, myalgia, running eyes, bleeding in skin and mucosa, rash, jaundice, myocarditis, meningitis, renal failure or pulmonary haemorrhagic symptoms.' Leptospirosis is a notifiable disease in the Netherlands, and human leptospirosis cases are registered by the public health services and the reference centre for leptospirosis in the Netherlands. For the use in this model, data from 1997 to 2015 from the two databases were compared and combined. Only cases were considered of which the location of infection could be determined from the patient history. The resulting list of human leptospirosis cases from 1997 to 2015 was used as the outcome variable of our model. The cases were aggregated over 4-digit postal code areas (PC4, approximately street level). This resulted in 3851 PC4's with no cases, 179 PC4's with one case, 20 PC4's with two cases, and three PC4's with three cases.

Data collection – Environmental variables: Environmental maps were obtained for use as covariates in the model. First, since there is a strong association between leptospirosis cases and (recreational) water, the presence of water was included using 'waterlayer' of the TOP500NL map [11]. Using the length of the waterways in a cell, this vector map was rasterized onto a 300×300 cell raster, covering the bounding box of the Netherlands in RD-coordinates, the national coordinate system of the Netherlands. The bounding box in these coordinates is bottom-left: $(0{,}3 \times 10^5)$, top-right: $(3 \times 10^5{,}6 \times 10^5)$. Only rivers and other waterways were extracted, not, e.g., lakes or ponds. These are included in the soil map which is described further on.

Second, a soil moisture map was used [12], with daily soil moisture for the year 2014. For each pixel, soil moisture was averaged over all days in the year. It was included for the relation between leptospirosis and water and rasterized as described above.

Third, estimates of rat density were included. In an ongoing control program of the muskrat population, by-catch of brown rats is registered because of animal welfare laws. This data was analyzed from 2009 to 2017 [13]. The reported number of captured brown rats in a 5-km square was adjusted by dividing it by the total number of hours that had been worked in that square. Almost all grid cells are square and have equal areas, thus we considered the variation in the total number of brown rats due to varying control area to be negligible. The control activities covered the majority of the Netherlands, with exceptions of a few grid cells, mainly in middle of the country near the largest national park, which are not likely to affect the results. The high data coverage made it possible to calculate a nationwide map on the local population densities of brown rats.

The final covariate included was soil type [14]. The variable soil type was rasterized into eight variables, each representing the fraction of the soil type within a cell: built-up area, clay, loam, spongy peat (an approximate translation of the Dutch term 'moerig'), peat, water, sand, and sandy clay.

In a final step, the surrounding countries plus the North Sea were masked and thereby removed from the spatial grid.

Predictive risk model: A random forest for classification was used for predicting human cases [15]. Covariates were included by averaging them over a 10 km buffer around each case PC4 location. The outcome metric was transformed into two categories: low (zero or one case in a grid cell) or high (two or three cases in a grid cell), as this was found to be more successfully predicted than a numerical outcome. The output variable is a probability which should be interpreted as 'the probability of a high incidence of human leptospirosis cases'.

There was a high imbalance in the data set: >4000 cells with low case count and 23 cells with high case count. Class imbalance can potentially lead to poor performance of a predictive model. Hence, we randomly downsampled to 23 cells of low incidence.

Predictors were checked for near-zero variance, indicating a signal which is too constant to be of any practical use, but none were found. Also, predictors were checked for correlations and it was found that 'spongy peat' was very highly correlated with 'moisture' (see supplementary material S1). Consequently, we removed 'spongy peat' from the list of predictors.

Random forest analysis was performed in R [16] using the randomForest package [17]. Model parameters were optimized by repeated cross-validation.

Field study

Site selection: The predictive risk map obtained from the random forest model was visually compared with actual disease incidences from 2009 to 2015. Four categories were distinguished: areas with many predicted cases and many patients ($pred^{+}pat^{+}$), areas with few predicted cases but many patients ($pred^{-}pat^{+}$), areas with many predicted cases but few patients ($pred^{+}pat^{-}$), and areas with few predicted cases and few patients ($pred^{-}pat^{-}$). Based on this comparison, three study sites close to recreational water were selected to test the hypothesis that addition of the prevalence of *Leptospira* spp. in brown rats would improve the predictive model. Location 1 (Appeltern) was a control area with a high predicted risk and relatively a high number of human leptospirosis cases ($pred^{+}pat^{+}$). Location 2 (Maastricht) had a low predicted risk but a high number of cases ($pred^{-}pat^{+}$). Location 3 (Roermond) had a high predicted risk but a low number of cases ($pred^{+}pat^{-}$) (see Figure 1 and supplement S2).

The animals: The study was approved by the Dutch Central Animal Experiments Committee (project number AVD3260020172104). In each location, at least 25 brown rats were captured. Rats were captured by employees of a professional rodent control company using snap traps with an eMitter alert system (Gorilla traps) baited with food. Thirty traps per study site were placed next to the water body, except for the second capture period in Roermond, when 15 traps were placed about 35 m from the water front. Trapping of rats continued till 25 rats were captured. The rat carcasses were stored at −20°C. During post mortem inspection, rats were weighted and kidney samples were collected and stored at −80°C until further processing. A small piece of kidney (10–30 mg) was collected, including medulla and cortex. DNA was extracted using the Fastprep-24 5 G (MP Biomedicals) and Lysismatrix D (MP Biomedicals) isolated using Magnapure (Roche). Testing of kidney samples for *Leptospira* spp. was performed by a Real-Time PCR for pathogenic leptospires, targeting the *LipL32* gene, as previously described [18]. Prevalence rates and binomial confidence intervals were determined using R version 3.5.1 [16].

Surface water: To test whether a high prevalence of *Leptospira* spp. infection in rats corresponds to contaminated surrounding surface water, surface water samples were collected at the three study sites. Ten samples of 1 L surface water were collected within 10 m of the three sites where rats were captured. Samples were frozen at −20°C until further processing. After thawing, water was filtered using membrane filters (Mixed Cellulose Ester) with a pore size of 0.45 µm (Merck). DNA was extracted from the filters by using the PowerWater DNA extraction kit (Qiagen, Hilden, Germany). To detect *L. interrogans* DNA, extracts were analyzed in triplicate using the method described by Ahmed et al. [18] on the CFX96 instrument (Biorad). PCR inhibition was assessed based on a control for successful DNA extraction and PCR amplification which is included in an assay [19] that was carried out in parallel. This multiplex assay detects signature sequences from

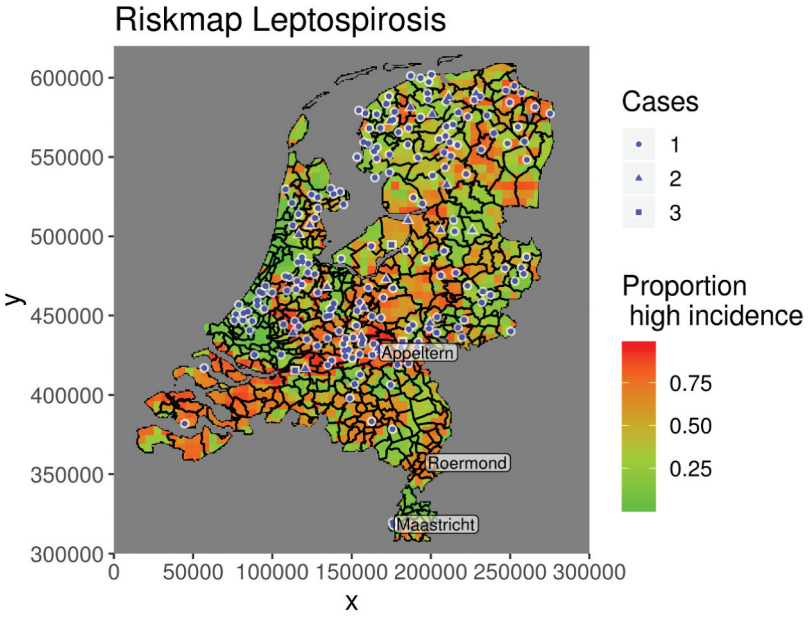

Figure 1. The colours in this leptospirosis risk-map represent the probabilities of realizing a high incidence of human leptospirosis cases. Overlaid are the actual human case locations, with the number of cases per PC4 area indicated by different shapes. The three field study areas are indicated. 'x' and 'y' are the coordinate axes of the RD_NEW (Amersfoort coordinates) national coordinate system.

Francisella tularensis, as well as from *Bacillus thuringiensis* spores which are spiked before DNA extraction to serve as internal extraction and amplification control.

Results

Development of the predictive risk map

The predictive risk map for leptospirosis, including the three regions where additional rats were captured and testing was performed, is shown in Figure 1. The final model had an overall error rate of 26%, which can be further explored using the confusion matrix (Table 1). Low incidences (22%) were better predicted than high incidences (30%). The four most important input variables for the model were (in order) water, clay, rat density, and sand. The presence of water (with an optimum of around 20% land cover) was by far the most influential. The influence of these four variables was summarized using partial dependence plots, depicting the contribution of each input variable to the outcome variable, which is 'the probability of a high incidence' (Figure 2a–d). The plots showed that different behaviours are possible. If a variable did not contribute to the risk, an effect of 50% would be seen (i.e. random chance). However, for water, there was clearly an optimal water coverage: around a fraction of 0.1, a rather narrow band of

Table 1. Results of the predictive model in a confusion matrix.

	Low incidence predicted	High incidence predicted	Classification error	95% Confidence Interval
Low incidence actual	18	5	22%	7–44%
High incidence actual	7	16	30%	13–53%

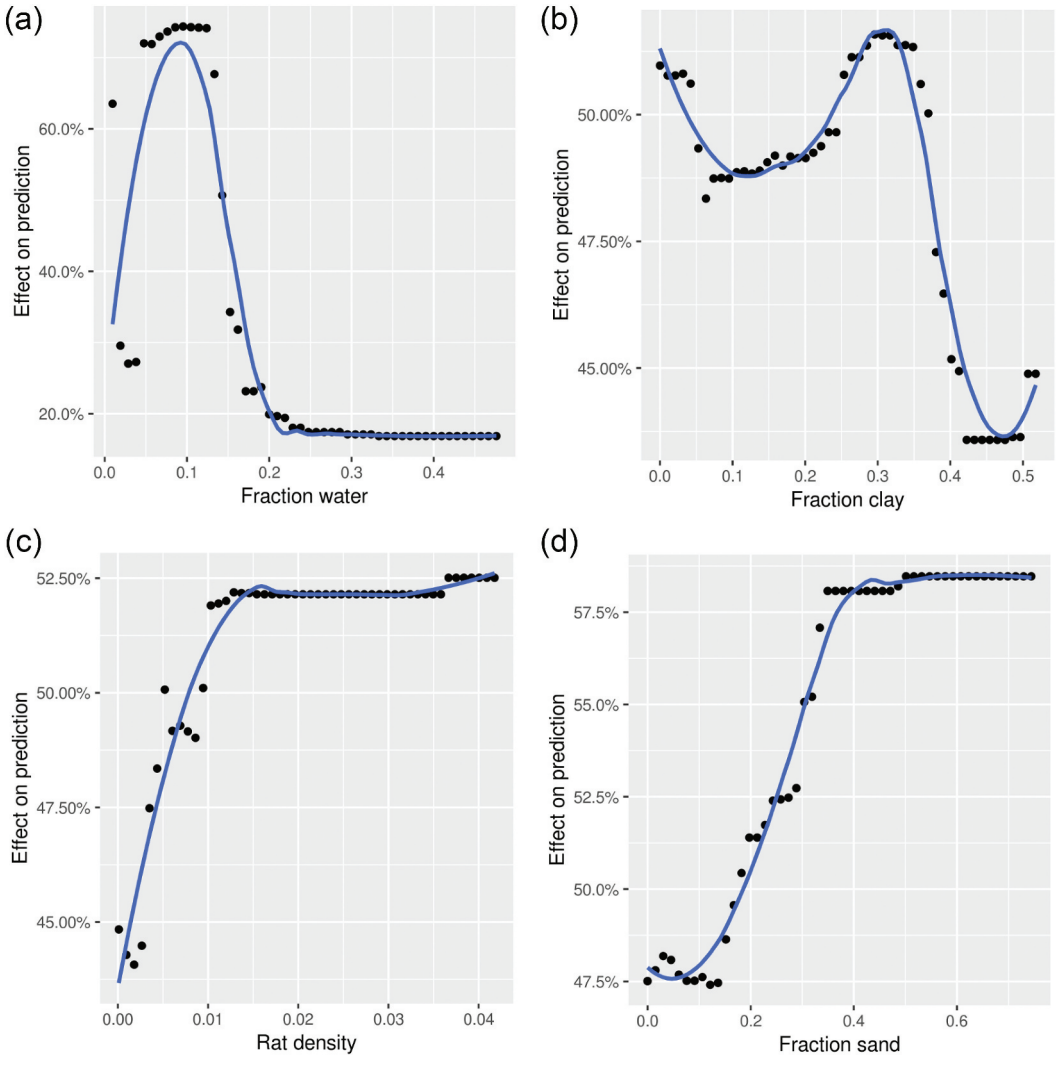

Figure 2. 2a-2d. Partial dependence plots of the four most influential variables, water (a), clay (b), rat density (c) and sand (d) showing the contribution to the outcome (probability of high incidence of human leptospirosis cases) as a function of the value of the predictor, averaged over all other variables. The dots are the 36 training data points, the blue line is a fitted non-parametric curve.

water coverage existed that contributed highly to leptospirosis incidence. Clay had only small, but erratic influence until around a fraction of 0.3, after which the contribution rapidly dropped. The rat density did not have a convex 'dip' at the beginning of the curve, meaning the influence of rat density rose immediately, until a maximum contribution was realised at approximately 0.015 rats per 5-km square/hour worked in that square. Sand had a minimum at about a fraction of 0.05 after which the risk increased with an increasing fraction of sand, until a plateau was reached around 0.4 where more sand did not make a difference anymore.

Validation of the risk map was performed in two ways. Firstly, the random forest method performs an internal validation on each tree of the forest individually, by splitting data into a 'training' and a 'testing' set, and performing predictions on the test set. The aggregated result of this cross-validation is shown in Table 1. Secondly, a qualitative validation can be performed visually by considering the risk map presented in Figure 1. It can be observed that high and low risk areas tend to cluster, and that they correspond well with the reported case data.

Rats

Rats were captured between December 2017 and the end of February 2018 (winter period). At location 3 (Roermond), only 14 rats were captured in this period. Therefore, capturing was continued in August 2018 at a nearby location, until 25 rats were reached. To correct for potential seasonal or geographical variation, the results of these two periods were analyzed separately.

At location 1 (Appeltern, pred$^+$pat$^+$), 16 out of 25 rats (64%) were found positive for *Leptospira* spp. At location 2 (Maastricht, pred$^-$pat$^+$) 25 out of 30 (83%) were positive. At location 3 (Roermond, pred$^+$pat$^-$), 7 out of 14 rats captured in winter were positive (50%), whereas 1 out of 11 rats captured in summer was positive (9%). The difference between the prevalence of Maastricht and Roermond (winter capture) was significant ($p = 0.03$, Fisher's exact test). The average weight of rats captured at location 3 in the winter was 237.3 g (range 182–377 g) and was higher than the average weight from the rats captured during the summer, which was 138.3 g (range 78–287 g) (Table 2).

Surface water

Surface water samples were collected in close proximity to where rats were captured (Supplement S1). In Roermond, water samples were collected during the first capture period. None of the 30 surface water samples were found positive for *Leptospira* DNA.

Discussion

Risk mapping can be a powerful research tool, not only to help direct surveillance and control programs but also to study what biological processes underlie transmission [20]. Using the developed predictive risk map for human leptospirosis incidence levels, a correlation was found of water, rat density, and sand/clay composition with leptospirosis risk. The predictive performance of the model is acceptable, with an overall 26% error rate. However, the data underlying the risk map have several limitations. First, the number of human leptospirosis cases is likely underestimated, since mild cases are often not recognized. Second, there may be a reporting bias between different areas in the Netherlands, as some areas are known as high risk areas, resulting in increased awareness amongst medical professionals.

An earlier study on spatial association of leptospirosis cases with environmental variables was performed by Rood et al. [10]. In concordance with the results of that paper, it was found in the current study that clay is a major driver. However, water bodies which were identified as highly contributing in our model are not explicitly found as risk factors in the Rood paper, possibly because their variable 'water banks' did not include recreational waters, lakes, and ponds. As already anticipated in the discussion of the Rood paper, a high relevance of rat presence was confirmed in the current work. Using a random forest technique has the advantage that it does not

Table 2. Overview of the results of the detection of *Leptospira* spp. in rats in the three study areas.

	Location	Category	Capture period	nr. of *Leptospira* positive rats/ total (percentage [95% confidence interval])	Average weight (range)
1	Appeltern	pred$^+$pat$^+$	2 December 2017–26 January 2018	16/25 (64% [43%-82%])	237.2 (113–462)
2	Maastricht	pred$^-$pat$^+$	26 January – 1 March 2018	25/30 (83% [62%-94%])	268.5 (36–424)
3a	Roermond	pred$^+$pat$^-$	17 January- 5 February 2018	7/14 (50% [23%-77%]) (winter)	237.3 (182–377)
3b	Roermond	pred$^+$pat$^-$	12 August – 16 August 2018	1/11 (9% [0.2%-41%]) (summer)	138.3 (78–287)

resort to a smoothing technique, such as the naïve Bayes smoother in the Rood paper. Furthermore, model interrogation is directly based on error-rates instead of significance scores (p-values). The model also accounts for non-linearity and interactions of the predictors. On the other hand, the handling of spatial autocorrelation is explicitly modelled in the Rood approach, while this is not (directly) possible using a random forest technique. Rather, spatial dependencies enter the random forest model via averaging covariates in buffers around pixels. Also, time dynamics is not easily incorporated using random forests. More advanced modelling techniques may be employed in future studies, for example, to include temporal dynamics and explicit modelling of spatial correlation, such as was done in a recently published stochastic spatially explicit model to forecast leptospirosis outbreaks [21].

Although the model performed acceptable, visual inspections revealed that at some locations there was particular strong misclassification. We hypothesized that the *Leptospira* spp. infection prevalence of the rat populations was an explaining factor for misclassification of the model and performed additional sampling in three selected rat populations. The *Leptospira* spp. prevalence varied from 9% to 83% in the investigated populations, but such large variation has been described before in other populations [22]. Overall, the prevalence of *Leptospira* spp. positive rats was higher than in previous field studies in the Netherlands [9]. The difference in *Leptospira* spp. prevalence at the two sites of location 3 (Roermond) during two seasons may be explained by spatial differences, but also by seasonal differences: the lower average weight of rats caught in summer can be indicative for a lower average age, and the probability of infection increases with age [23]. For the comparison between locations, it was decided to take into account only those captured at location 3 during winter season.

The highest prevalence of infected rats (83%) was found in the $pred^{-}pat^{+}$ area, whereas the lowest prevalence (50% when considering only the winter) was found in the $pred^{+}pat^{-}$. This suggests that *Leptospira* spp. infection prevalence of rats could be a valuable variable for the predictive model. To substantiate this suggestion, a larger scale study would be necessary, as in the current study the sample size is too small to draw firm conclusions. However, the techniques used for assessing infection in rats are quite laborious to implement on a large scale. Serologic assays are not an alternative, because *Leptospira* spp. infection in rats causes asymptomatic infections with inconsistent immune responses [24], that may also be directed against nonhomologous serovars other than those that they are infected with [25].

Therefore, the use of water samples was explored for the detection of *Leptospira* spp. as an alternative for the testing of rats. Traditional analysis of water through culturing is also very time and effort consuming, but advances in molecular techniques detecting DNA of pathogens in water have made such analyses more applicable [26,27]. To test whether the detection of *Leptospira* spp. DNA in surface water would be a reliable alternative for estimating infection risk of rats or humans in practice, surface water samples were tested that had been collected close to the capture locations of rats. However, none of the 30 surface water samples was positive.

In this study, *Leptospira* spp. in surface waters was no indicator for infection risk of rats. In contrast, Casanovas-Massana et al. [28] did find a correlation between leptospirosis incidence in humans and *Leptospira* spp. concentrations in water. However, their study was done in a tropical environment and focused on sewage water, whereas the samples in the current study were collected in a temperate climate in winter and from surface water without input from untreated sewage.

In conclusion, a predictive risk map for human leptospirosis incidence levels was developed and assessed, including various environmental factors and rat density as variables. The inclusion of rat density improved the classification of the model. Results of the field study suggested that *Leptospira* spp. infection prevalence in rats may explain model misclassifications and give inspiration for further research. In this study, detection of *Leptospira* DNA in surface water was not a good alternative for the testing of rats, but conditions for successful use of this method could be further explored in future studies.

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Disclosure statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

MM, JvdG and AS designed the study; MM, AdV and TC performed necropsies; AdV performed *Leptospira* detection in rats; MK and IJ performed *Leptospira* detection in surface water samples; AS developed the predictive risk map. All authors read the manuscript and agreed with publication.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author (MM) upon reasonable request.

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