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The association between natural drinking water sources and the emergence of zoonotic leptospirosis among grazing beef cattle herds during a human outbreak

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ABSTRACT

Leptospirosis is a zoonotic bacterial disease associated with water abundance in tropical and temperate climate zones. Bacterial spread may also occur in dry and warm weather conditions when humans and animals are forced to share depleted water sources. In such settings, farm animals such as beef cattle, which may be present in large numbers in natural water sources, can play a major role in disease spread. However, the risk factors for their infection and the potential control measures to prevent the disease spread have not been adequately studied.

In the face of an emerging human leptospirosis outbreak in the dry and warm Israeli 2018 summer, we tested seropositivity to *Leptospira* serovar Pomona in grazing beef cattle and wild boars located in proximity to the contaminated streams. Additionally, we used the natural setting of the outbreak to identify risk factors for seropositivity in beef cattle.

We found high seropositivity to serovar Pomona in grazing beef cattle (233/845), and in wild boars (7/13). Seropositivity was significantly associated with beef cattle drinking from natural water sources compared to beef cattle drinking from water troughs with fresh water supply (Multivariable logistic regression; odds ratio = 18.6, 95% confidence interval = 3–116, p-value<0.01).

One Health approach is necessary for mitigating zoonotic *Leptospira* infections, in which interactions between humans, animals, and the environment play a major role. As the global warming crisis results in severe climate changes, dry and warm weather conditions may become more common worldwide. Under such conditions, reducing inter-species interactions in contaminated natural water sources is essential for protecting public health. Our study demonstrates the role of natural water as a source for beef cattle infection and disease spread. Furthermore, we suggest using water troughs with freshwater supply for preventing future outbreaks in animals and humans in such settings.

1. Introduction

Leptospirosis is a globally distributed zoonotic disease caused by spirochetes of the genus *Leptospira* [1]. Its annual global burden is estimated at over one million human cases and 60,000 deaths [2].

Leptospira infections may occur through direct or indirect contact with urine or tissues of infected animals [1]. Infections with host-adapted serotypes, such as serovar Hardjo in cattle or serovar Pomona in pigs, may go unnoticed or, result in chronic infections, infertility, abortions, stillbirths, and premature birth of weak live offsprings [3]. Infected animals may shed bacteria in body secretions for prolonged periods [4]. Infections with serotypes not adapted to the host, such as serovar Pomona in cattle, result in acute sickness characterized by clinical signs such as pyrexia, sudden onset of agalactia, infertility, and

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abortions. The shedding of bacteria may be transient in these acute cases [5].

Human infections could occur either sporadically or in the context of an outbreak. Infections may be acquired through occupational, recreational, or avocational activities [6] involving exposure to contaminated urine or other body secretions of infected mammals, either directly or via contamination of soil or water [4].

Leptospirosis outbreaks were described in tropical [7–12] and temperate [13,14] climate zones. These were often associated with floods or increased rainfall that resulted in the elevation of the water table, allowing favorable conditions for the spread of the bacteria [14]. Warm weather conditions were also suggested as a possible risk for leptospirosis outbreaks due to the increased interaction between humans and animals sharing the depleted water sources [6,15], yet such outbreaks and the risk factors for disease spread were less often described. With the climate changing as a result of the global warming crisis, such conditions may become more common [15]. The nature of the disease and the role of the environment in transmission necessitate the use of an integrative, holistic and proactive one-health approach, which may be essential to confronting emerging and re-emerging zoonotic pathogens [16].

In Israel's warm and dry Mediterranean temperate climate, leptospirosis is mainly sporadic in humans, with approximately 10 annual cases caused by various serotypes from 2001 to 2017 (Ministry of Health; www.gov.il/he/Departments/dynamiccollectors/weekly-epidemiological-report; in Hebrew) and a single outbreak that involved serovar Hardjo in 2002 [17].

Since 2009, a gradual increase in the number of cases caused by serovar Pomona was observed in various animal species (i.e., cattle, dogs, swine and horses; Israeli Veterinary Services (IVS) annual reports; www.moag.gov.il/vet/dochot-shnatiim; in Hebrew) without a corresponding increase in the incidence of human cases. However, during the summer of 2018, a large outbreak of leptospirosis occurred in northern Israel. Over 600 leptospirosis cases were suspected among individuals with a history of recreational activities in certain streams and natural pools in the Golan Heights. Testing of confirmed cases identified serovars Pomona and Balcanica as plausible causative serovars [18]. Among these cases were travelers later diagnosed as positive in the United States [19] and Europe [18]. During the outbreak, water sources were found to be highly contaminated with fecal material of animal origin [18], yet the exact source has not been entirely ascertained.

At the time of the large-scale human outbreak, abnormal clusters of third-trimester abortions among pregnant cows and heifers (up to 50% of the pregnant animals) were reported from two farms of free-range beef cattle herds in the Golan Heights. *Leptospira* infection with serovar Pomona was suspected according to the Kimron Veterinary Institute (KVI) routine screening tests in cases of abortion that included micro-agglutination tests (MAT) using an antigen panel of eight serovars (Canicola, Pomona, Tarassovi, Hardjo, Grippotyphosa, Bratislava, Ballum and Icterohaemorrhagiae).

In face of the large scale human outbreak and the possible spread of the pathogen in cattle, we conducted an epidemiological investigation in the drainage basin region where the 2018 outbreak occurred to determine the seroprevalence of serovar Pomona in wild boars (*Sus scrofa lybicus*) and grazing beef cattle herds and to evaluate the risk factors for infection of the latter.

2. Materials and methods

2.1. Study population and sample size

An epidemiologic investigation targeting beef cattle herds and wild boars in the Golan Heights, in the vicinity of the 2018 outbreak was conducted [18]. In this area, natural bodies of water and their drainage basins situated within nature reserves are used for human recreational activities, grazing of beef cattle herds, and wildlife activity. These shared bodies of water may serve as a sole source of drinking water for both domestic and wild animals.

During the period of August 16–October 4, 2018, blood samples were collected from 848 cattle belonging to 29 herds from 11 beef cattle farms. Cattle farms were selected for this study based on their proximity to the infected water streams identified during the human outbreak [18]. Cattle were divided by farm owners into herds according to farm management considerations such as age, stage in pregnancy, etc. Herds were grazing on separate areas that were surrounded by fences to prevent the mixing of animals from different herds. The available water sources (i.e. water troughs or natural water streams) varied between the grazing areas. The IVS chief veterinary officer held the epidemiological investigation to evaluate the prevalence of leptospirosis in the herds and to devise a control strategy to prevent further spread. Following Israeli law, the sampling was conducted according to the ethical standards accepted by the IVS for outbreak investigations. Ten to 45 samples (median = 30) were collected from 1 to 9 herds (median = 1) in each farm. Epitools epidemiological calculators [20] were used to determine the required sample size within herds for estimating disease prevalence. For this purpose, the apparent prevalence within each herd was estimated at 10%. A sample size of 35 animals was therefore required to estimate the true within-herd seroprevalence of Pomona serovar with a 95% confidence interval ($CI_{95\%}$) and precision of 10%. Within each herd, animals were selected randomly and blood samples were collected from the coccygeal vein into marked vacuum tubes containing a clot activator. The samples were kept refrigerated in a cooler until serum separation was performed in the laboratory. Information on animal age, sex, farm and herd sizes (i.e. number of animals), grazing area locations and the drinking water sources serving each herd (i.e. streams and natural pools versus fresh water from water troughs) was recorded at each farm.

Blood samples were collected from wild boars ($n = 13$) in the same area on September 3, 2018 by the Israel Nature and Parks Authority (INPA) as part of the Israel Wildlife Disease Surveillance Program (IWDS), in which samples of multiple wild animal species are collected and screened for certain diseases. The wild boars were killed according to the ethical standards accepted by the Israeli law, and blood samples were collected directly from the heart into vacuum tubes and handled as described above.

During the same period, blood samples were collected from cattle and wild boars grazing in the Golan Heights at locations distant from the outbreak area. Thirty and 19 samples were collected from two beef farms. Eight blood samples were obtained from wild boars. These samples were analyzed separately from those collected in proximity to the 2018 outbreak (see below).

2.2. Serological testing

Micro-agglutination tests for detection of *Leptospira* antibodies were performed at the KVI diagnostic laboratories, Israel. Samples that had undergone considerable hemolysis, for which MAT could not be conducted, were removed from the analysis ($n = 3$ beef cattle and $n = 2$ wild boars). Sera were diluted 1:25, and then two-fold up to an end-point of 1:102,400 in phosphate-buffered saline containing 0.2% formaldehyde. They were then tested for serovar Pomona against fresh-grown *Leptospira* antigens (NVLS, APHIS, USDA) in 0.2% formaldehyde. MAT results were interpreted as previously described [21]. Dilutions of 1:50 to 1:400 were examined, and titers of 1:100 and above were regarded as positive, per World Organization for Animal Health (OIE) recommendations [22]. In addition, data were analyzed using a titer of 1:200 as the cutoff for seropositivity (see below).

2.3. Data analysis

Grazing locations of the farms and herds and approximate locations of the wild boars were mapped using ArcGIS 10.6 (ESRI, Redlands, CA,

USA). We calculated the prevalence of seropositivity in each herd, and the proportion of seropositive wild boar samples. Cattle age was categorized to animals younger than 24 months or adult; farm and herd size were divided to categories (i.e. large or small) using cutoffs of 700 and 200 animals, respectively; farm and herd grazing area sizes (in square kilometers) were calculated using ArcGIS 10.6 (ESRI, Redlands, CA, USA), and then divided to categories (i.e. large or small) using cutoffs of 17- and four-square kilometers, respectively. Herds were also categorized according to the available water drinking sources in the grazing areas. The water source of a single herd of pregnant calves ($n = 45$), which had access to both natural water sources and water troughs, was regarded as a natural drinking source in the analysis. Associations between the animals' seropositivity and single covariates (i.e. 'Age', 'Sex', 'Farm size', 'Farm grazing area', 'Herd size', 'Herd grazing area' and 'Drinking water source') were investigated using chi-square tests. Only factors significantly associated (p-value <0.1; Fisher's exact test) with serovar Pomona seroprevalence status were used for fitting a multivariable model. Collinearities between variables to be included in the multivariable model were tested. For fitting a multivariable model, we examined mixed-effects logistic regression (GLMER) with 'Farm' or 'Herd' as random effects and a generalized linear model (GLM) and the preferred model was selected based on the Akaike Information Criterion (AIC). A stepwise backward selection method was used to reduce the risk of type II error (i.e. falsely failing to reject the null hypothesis). A probability of 0.05 was defined for the removal of variables. Data summarization and statistical analysis were performed using the dplyr v1.0.2 [23], stringr v1.4.0 [24], lme4 v1.1.23 [25] and broom.mixed v0.2.6 [26] packages in R software v4.0.2 [27]. If not stated otherwise, a significance level of $\alpha = 0.05$ was applied.

3. Results

In face of the large scale human outbreak and the detection of serovar Pomona in two cattle herds with clusters of abortions during late pregnancy at that area (Fig. 1; Farms 1 and 11), an epidemiological investigation was initiated and seropositivity for serovar Pomona was tested among herds from these and other farms located in the Golan Heights in close proximity to the 2018 outbreak. None of these farms was previously vaccinated against serovar Pomona. A commercial vaccine (Spirovac®, Zoetis, USA) against serovar Hardjo was the only available vaccine at the time. This vaccine was used in young heifers before breeding (12–18 months old) in cases of increased incidence of abortions in the herd. The seropositivity rate for serovar Pomona was found to be 233/845 beef cattle (27.6%, $CI_{95\%}$ = 24.6–30.1%) (Fig. 1). In the univariable analysis, positive associations were found between seropositivity and all tested covariates (i.e. 'Age', 'Sex', 'Farm size', 'Farm grazing area', 'Herd size', 'Herd grazing area' and 'Drinking water source'; Table 1, $p < 0.1$).

Collinearity was found between the variables 'Farm size' and 'Farm grazing area' and the latter was removed. In the multivariable analysis, the GLMER model was preferred over the GLM based on the AIC values. 'Herd' was used as the random variable in the model. Adding 'Farm' as a random variable did not change the model estimates, yet the AIC value was increased; therefore, this variable was omitted from the final model. In the final model, 'Herd size' and 'Herd grazing area' were included, albeit were not significant; only 'Drinking water source' remained statistically significant (Table 2, Odds ratio 18.6 CI 3.00–116, $p < 0.05$).

To resolve potential disagreements regarding the cutoff for seropositivity, data were also analyzed treating a titer of 1:100 ($n = 23$ samples) as seronegative and similar results were obtained (data not shown).

In addition, detailed information on animal movements was available for a single farm, of which nine herds were included in this study (Fig. 1; Farm 1). In this farm, bulls intermingled with three of the cowherds for breeding purposes before being transferred to a separate grazing location on June 1, 2018. None of the bulls (0/20) was found seropositive. However high seroprevalences were found in the cow herds (52–90%) when examined on August 29, 2018. These cow herds were relying mainly on natural drinking water sources, while the bulls were drinking from water troughs available in the separate grazing area (Fig. 1; Farm 1).

Seropositivity for serovar Pomona was found in 7/13 wild boars (54%, $CI_{95\%}$ = 25.1–80.8%) located in the Golan Heights in close proximity to the 2018 outbreak (Fig. 1), however, no clinical signs of leptospirosis, such as icterus and hemoglobinuria, were apparent.

All sera obtained from beef cattle ($n = 49$) and wild boars ($n = 6$) that were located in the Golan Heights in areas distant from the reported human outbreak were found to be negative (Fig. 1).

4. Discussion

We described a high seroprevalence of antibodies against serovar Pomona among animal hosts located in the Golan Heights at the time of a large-scale human outbreak under dry and warm climate conditions following several consecutive years of drought in Israel [18]. Moreover, in the natural setting of the outbreak, we identified drinking from natural water sources as the main risk factor for seropositivity in beef cattle. This may suggest that providing water troughs with fresh drinking water can assist in controlling disease spread. Such a control measure will reduce the dependency of grazing beef cattle on natural water sources and, therefore the risk for disease spread as it will reduce contamination from animal excretions in the streams and the possible encounters with wild animals. The potential importance of this control measure in disease prevention may also be reflected by the low seroprevalence (10%) found in a group ($n = 45$) of pregnant cows which had access to natural water sources and water troughs with fresh water in their grazing area.

Serovar Pomona was consistently detected in the MAT against eight *Leptospira* serovars as part of the initial screening tests conducted by the KVI diagnostic laboratories in the first cases of abortion in cows from the index farms (Fig. 1; Farms 1 and 11). This serovar, which was increasingly detected in animals in Israel since 2009, was also one of two serovars suspected of causing human infections during the 2018 outbreak (the other was Balcanica) [18], and therefore it became the focus of the seroprevalence survey conducted here.

With the exception of one anecdotal report of a seropositive beef bull on the northern slopes of the Golan Heights in 2017 (IVS Annual Reports; https://www.moag.gov.il/vet/dochot-shnatiim/Pages/default.aspx; in Hebrew), this is the first detection of this serovar in the Golan Heights plateau. The lack of previous evidence of this serovar in the area may suggest that the beef cattle grazing in the Golan Heights were infected shortly before the human infections during the 2018 leptospirosis outbreak. This is further supported by the lack of serological evidence for infection in bulls that intermingled with cow herds that later demonstrated high seroprevalence ranging from 52 to 90%. These bulls were placed with the cows for breeding purposes; frequent animal-animal encounters and sharing of resources were therefore highly likely. The bulls were transferred to a separate grazing area on June 1, 2018, approximately a month before the first human case was detected [18]. The introduction time of the pathogen to the beef cattle is therefore likely dated between transferring the bulls to a separate grazing area and the first cases of abnormal abortion clusters in cows.

The significant association of seropositivity with drinking from natural water sources as opposed to water troughs (Odds ratio = 18.6) points to the main potential source of infection for beef cattle. Contaminated water was previously described as a source of infection with *Leptospira* in other species [28]. The bacteria enter the body through small cuts, mucous membranes, or wet skin [1]. In opposition to our findings, water troughs were previously suggested as a possible risk factor for *Leptospira* serovar Hardjo infection in cattle [29] and sheep [30]. However, in these studies the water supply originated from streams and creeks in that area [29,30], which were likely to be contaminated. Here, fresh water was supplied either through the general

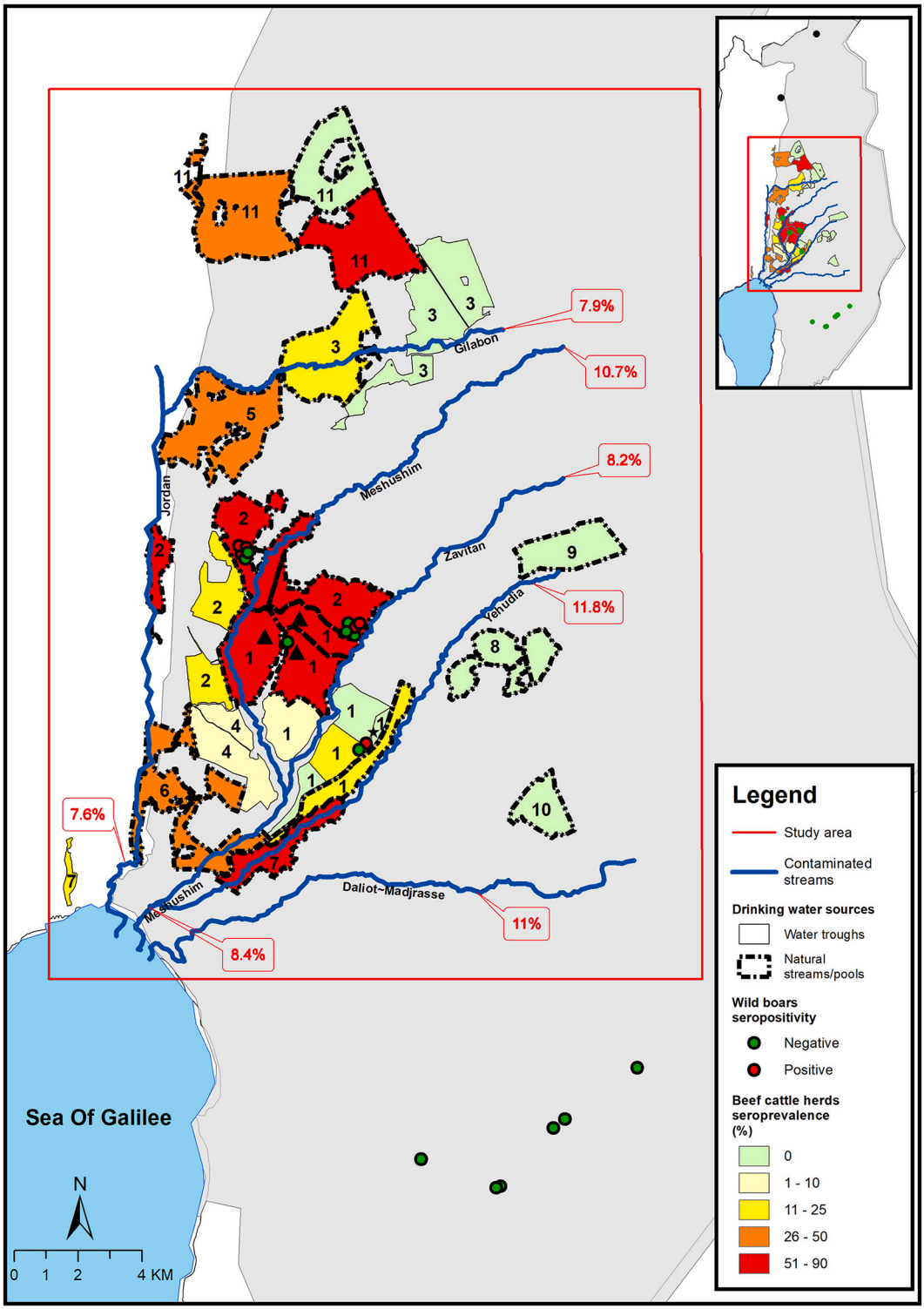

Fig. 1. Seroprevalence of Pomona serovar in wild boars and beef cattle herds located in high proximity to the 2018 leptospirosis human outbreak (red square) in the Golan Heights region (grey), Israel. Additional wild boars and beef cattle farms that were sampled are presented outside of the boxed area. Red and green circles indicate the approximate locations of the seropositive and seronegative wild boars, respectively (samples collected at the same location were manually shifted to prevent overlap and allow better visualization). Polygons indicate the grazing area of the beef herds, and the numbers indicate the different farms. Seronegative herds are colored in green, and warm colors indicate seropositivity (detailed seroprevalence data is included in Supplementary Table S1). An asterisk indicates the location of the bull herd from farm #1, and black dots indicate the three cow herds in which the bulls were placed for breeding purposes until June 1, 2018 (see text for details). The grazing areas of herds drinking from natural water sources are highlighted with a surrounding broken line. In addition, the percentage of positive human cases during the 2018 outbreak with a potential exposure to one of the water sources [17] are indicated in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Univariable risk factor analysis for *Leptospira* serovar Pomona seropositivity in grazing beef cattle located in the Golan Heights in close proximity to the 2018 human leptospirosis outbreak.

Risk factors	Odds ratio ($CI_{95\%}$[1])	Number of seropositive/ total (%)		*p* value [2]
		Exposed	Not exposed	
Age (Adult vs. Young)	2.3 (1.33–4.22)	216/734 (29)	17/111 (15)	<0.01
Sex (Female vs. Male)	14.91 (2.48–606.41)	232/807 (29)	1/38 (3)	<0.001
Farm size[3] (Large vs. Small)	0.54 (0.39–0.74)	131/563 (23)	102/282 (36)	<0.001
Farm grazing area[3] (Large vs. Small)	1.64 (1.12–2.44)	189/632 (30)	44/213 (21)	<0.05
Herd size[3] (Large vs. Small)	0.31 (0.19–0.5)	23/182 (13)	210/663 (32)	<0.001
Herd grazing area[3] (Large vs. Small)	2.78 (2.02–3.86)	146/376 (39)	87/469 (19)	<0.001
Drinking water source (Natural vs. Water troughs)	15.5 (8.63–30.24)	220/539 (41)	13/306 (4)	<0.001

[1] 95% confidence intervals.
[2] Fisher's exact significance level.
[3] Cutoffs of 700 and 200 animals were used for categorizing herd and group size variables, respectively. Cutoffs of 17 and four square-kilometers were used for categorizing farm and herd grazing area variables, respectively.

Table 2
Multivariable risk factor analysis for *Leptospira* serovar Pomona seropositivity in grazing beef cattle located in the Golan Heights in close proximity to the 2018 human leptospirosis outbreak.

Risk factors	Odds ratio ($CI_{95\%}$[1])	Sig.
Herd size[2] (Large vs. Small)	0.14 (0.02–1.31)	0.08
Herd grazing area[2] (Large vs. Small)	3.94 (0.74–21.1)	0.11
Drinking water source (Natural vs. water troughs)	18.6 (3.00–116)	<0.01

[1] 95% confidence intervals.
[2] Cutoffs of 200 animals and 4 km^2 were used for categorizing the herd size and area variables, respectively.

water supply (i.e. potable water) or directly from the origin of the spring. Contamination of these water sources was, therefore, less likely.

Even though all herds were likely to encounter potentially infected wild boars in the grazing areas, herds relying solely on natural water sources were likely to have more frequent encounters. The frequency of such encounters was likely aggravated due to the depletion of the water sources due to consecutive years of drought, which has also likely to result in higher concentration of fecal indicator bacteria in the contaminated water.

Whether the introduction of serovar Pomona to the Golan Heights Plateau was caused by wild boars or other wild or domestic animals has yet to be determined. However, regardless of their role in the introduction of the serovar, infected wild boars may play an important role in propagating the disease, as adapted hosts are likely to shed bacteria intermittently for long periods [31]. In non adapted hosts, such as cattle infected with serovar Pomona, bacterial shedding is often short [31]. However, the prominence of clinical signs in the acute phase of the disease in many animals probably contributed significantly to the contamination of water sources that led to the human infections during the 2018 outbreak [18]. The reduced water flow in the area secondary to drought probably contributed to increased concentrations of bacteria in water, capable of causing human infection upon recreational exposure. The global abundance of wild boars and their frequent encounters with domesticated livestock species [32] may result in a higher frequency of such scenarios in the future.

At the time of the 2018 outbreak, in order to prevent further spread of the disease, the IVS began to implement mandatory vaccination of grazing beef cattle herds in the Golan Heights region using inactivated commercial vaccines that include serovars Canicola, Pomona, Hardjo, Grippotyphosa and Icterohaemorrhagiae (Spirovac® L5 Zoetis, USA or Lepto Shield™, Elanco, USA). In addition, government funds were provided for adding water troughs in the grazing areas to reduce the dependence of the herds on natural water sources and the frequency of encounters with wild animals. The preliminary results of this study which were available at the time, supported these measures, and the final analysis presented here reinforces the potential importance of future application of such measures to minimize the risk of spreading this pathogen and protecting public health.

The main clinical sign that was identified during the outbreak in beef cattle was abortions during late pregnancy. Such clusters of abortions are an unusual event in the herds and indicate the severity of the infection. Given the wild nature of the free-roaming grazing beef cattle, detection of other clinical signs of acute sickness, such as pyrexia and sudden onset of agalactia [5], can be difficult. In addition, due to the immediate metaphylactic antibiotic treatment (with antibiotics such as streptomycin) in infected herds to avoid additional abortions, and vaccination (shortly after the serum samples collection), there was not sufficient time for the development of additional clinical signs in infected beef cattle.

In this study, MAT were used for the detection of infection with serovar Pomona. MAT hold high specificity and are widely adopted for *Leptospira* infection detection in both humans [33] and animals [22]. The test sensitivity is low (may be lower than 50%) for infection detection in individual animals, especially for detection of chronic infections with host-adapted serovars. However, the approach of testing of 10 animals or at least 10% of the herd, whichever is greater, is regarded as having high sensitivity for detection of infection at the herd level [22]. Moreover, a possible limitation of the MAT is cross-reactivity between antibodies of serovars [22]. This may also be the case for antibodies induced by commercial vaccines used in this study. As MAT were conducted primarily against serovar Pomona, false identification of Pomona infections due to previous vaccinations or cross infections with other *Leptospira* serovars may have occurred. However, the probability of such misidentification is less likely due to the following: a) The described outbreak in beef cattle and wild boars was detected at the time of a large human outbreak, and the animals were located in high proximity to the human cases, in which serovar Pomona was mainly detected; b) At the onset of the outbreak in the beef cattle herds, only antibodies against serovar Pomona were found at the KVI routine screening tests that included MAT using an antigen panel of eight serovars (See above); c) Before the described outbreak, vaccination against serovar Hardjo was applied only to heifers before breeding (see above), however, seropositivity was higher in adult cattle in comparison to young (Table 1).

The presented study further emphasizes the importance of a one health approach to prevent the spread of emerging zoonotic pathogens. We demonstrate here how an environment with a dry and warm climate can set the ground for a large-scale infection of animals (domestic and wild), which in turn may result in an outbreak in humans. Moreover, our experience with the 2018 human leptospirosis outbreak and the current analysis results demonstrate the importance of performing routine surveillance of pathogens of public health importance among livestock and wildlife. Timely detection of the introduction and dissemination of zoonotic pathogens to discrete geographic regions could assist in understanding the factors contributing to disease emergence and identifying potential risks at the human-animal-environment nexus, which could be further assessed and managed.

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Data availability

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

CRediT authorship contribution statement

Lior Zamir: Conceptualization, Formal analysis, Data curation, Investigation, Visualization, Writing – original draft. **Miri Baum:** Investigation. **Svetlana Bardenstein:** Resources. **Shlomo E. Blum:** Resources, Writing – review & editing. **Jacob Moran-Gilad:** Writing – review & editing. **Michal Perry Markovich:** Methodology, Writing – review & editing. **Roni King:** Investigation, Project administration. **Roi Lapid:** Investigation. **Fares Hamad:** Investigation. **Boris Even-Tov:** Investigation, Project administration. **Ehud Elnekave:** Formal analysis, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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