

## ORIGINAL PAPER

# Large-scale application of highly-diluted bacteria for Leptospirosis epidemic control

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**Background:** Leptospirosis is a zoonotic disease of major importance in the tropics where the incidence peaks in rainy seasons. Natural disasters represent a big challenge to Leptospirosis prevention strategies especially in endemic regions. Vaccination is an effective option but of reduced effectiveness in emergency situations. Homeoprophylactic interventions might help to control epidemics by using highly-diluted pathogens to induce protection in a short time scale. We report the results of a very large-scale homeoprophylaxis (HP) intervention against Leptospirosis in a dangerous epidemic situation in three provinces of Cuba in 2007.

**Methods:** Forecast models were used to estimate possible trends of disease incidence. A homeoprophylactic formulation was prepared from dilutions of four circulating strains of Leptospirosis. This formulation was administered orally to 2.3 million persons at high risk in an epidemic in a region affected by natural disasters. The data from surveillance were used to measure the impact of the intervention by comparing with historical trends and non-intervention regions.

**Results:** After the homeoprophylactic intervention a significant decrease of the disease incidence was observed in the intervention regions. No such modifications were observed in non-intervention regions. In the intervention region the incidence of Leptospirosis fell below the historic median. This observation was independent of rainfall.

**Conclusions:** The homeoprophylactic approach was associated with a large reduction of disease incidence and control of the epidemic. The results suggest the use of HP as a feasible tool for epidemic control, further research is warranted. *Homeopathy* (2010) 99, 156–166.

**Keywords:** Homeoprophylaxis; Prevention; Leptospirosis; Epidemics; Cuba

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## Introduction

Leptospirosis is a serious disease caused by infection with pathogenic strains of the Gram-negative bacterium *Leptospira* spp. In recent years, Leptospirosis has emerged as one of the most important zoonotic diseases worldwide

and a severe health problem in developing countries and the tropics.<sup>1-3</sup> According to antigenic determinants, *Leptospira spirochetes* are classified into 25 serogroups and over 250 serovars that affect almost all mammals. Human infection usually occurs when contact with the urine of domestic and wild animals (mainly rodents, dogs, pigs and horses) which are natural bacteria reservoirs secreting spirochetes in the urine, the animal vectors often remain symptom free.<sup>4</sup> Infection occurs through the mucosa or open skin lesions and the contact urine contaminated water.<sup>5-7</sup>

Under normal conditions, Leptospirosis is an occupational disease affecting individuals living in rural regions, mainly farmers involved in agriculture or animal breeding which are considered the main at-risk groups. However, an increasing number of Leptospirosis infections in urban areas and among adventure travellers practising water-sports has been reported in recent years.<sup>8-11</sup> Leptospirosis epidemics are a greater concern in developing countries where poor sanitary conditions, health structures, medical awareness and misdiagnosis have contributed to keep Leptospirosis as a major, but poorly recognised, threat. In tropical countries, the incidence of Leptospirosis is closely related to rainfall and flooding seasons when ecological conditions are favourable for the transmission of zoonotic diseases.<sup>1,2</sup> When infected urine contaminates water reservoirs, the bacteria can survive for long periods in water at neutral pH.

The symptoms caused by Leptospirosis infection are extremely variable and potentially dangerous, they include meningitis, pneumonitis, hepatitis, nephritis, mastitis, myocarditis, haemorrhagic crisis and multi-organ failure.<sup>12</sup> The broad spectrum of symptoms caused by Leptospirosis infection frequently leads to misdiagnosis, incorrect selection of medical treatment and high mortality rates, especially in regions where other infectious diseases with overlapping symptoms are also prevalent (eg, Dengue fever).<sup>13,14</sup>

The laboratory tests for Leptospirosis diagnosis are mainly based on demonstration of spirochetes in clinical samples (blood, urine and organ samples), or detection of serum antibodies. Leptospirosis cultures from clinical samples grow very slow and are a very late confirmatory method that should not be used to decide medical treatments.<sup>7,12</sup> Antibody detection by Indirect Hemagglutination Assay (HIA) has been considered the 'gold' standard for early diagnostic serology, although antibodies are often not detected in early phases of infection and the presence of antibodies is not a direct predictor of infection in endemic areas. The detection of bacterial DNA in blood samples by PCR is a sensitive and rapid method to confirm the infection but is not widely available and mostly limited to regional labs.<sup>15</sup>

Given the difficulties of confirming Leptospirosis infection, medical awareness and appropriate management of suspicious patients are key aspects to decreasing mortality rates. However, disease control strategies should be based mainly on prophylactic approaches addressing immune protection, transmission chain disruption and risk amelio-

ration. Among the most commonly used prevention alternatives, chemoprophylaxis has been demonstrated to be effective in outbreak control. However, considering the short half life of doxycycline in blood stream (18 h), its prophylactic effect is limited and unfeasible for large groups at risk in endemic areas.<sup>16,17</sup> In addition the effectiveness of doxycycline prophylaxis after severe climatic phenomena has still to be demonstrated.<sup>13</sup> The control of animal vector represents another strategy to disrupt the transmission chain but it has to be effective and sustained to significantly decrease infection risks. In view of the diversity of vector animal species and complexity of the ecosystems, especially after the severe damage caused by natural disasters, vector control is still far from being a realistic alternative.

Vaccination represents, to date, the most effective option for disease control despite the fact that Leptospirosis vaccines are not widely available.<sup>18</sup> vaxSpiral<sup>®</sup> is the commercial name of the only three-valent Leptospirosis vaccine available in the market. It is a whole cell inactivated preparation developed and produced at Finlay Institute, Cuba.<sup>19</sup> vaxSpiral<sup>®</sup> demonstrated a 78.1% efficacy and good safety profile in clinical trials conducted in Cuba has been included in the national immunization program since 1998 for immunization of individuals over 15 years old in at-risk groups (mainly farmers and animal breeding workers).<sup>20-24</sup> However, because of the time needed to complete the immunization schedule and to reach high coverage the effects of vaccination on decreasing the incidence are significant only over the long term.<sup>24</sup> Particularly in endemic regions suffering sudden-onset epidemics, the effect of vaccination programmes can be very slow because of the high circulation of pathogens and the continuous modification of group at risk.<sup>25</sup>

There are few published trials of disease control using homeopathic medicine as preventive method, homeoprophylaxis (HP).<sup>26-29</sup> These approaches involve the use of highly-diluted and succussed (potentised) material from different sources including plants, animals, minerals and bacteria. HP has been used in epidemic situations since 1798 and as an alternative to routine vaccination programs for the prevention vaccine-preventable and non-preventable diseases.<sup>27,30-33</sup> Potentised pathogens or disease products known nosodes or biotherapies have been reported to be effective in controlling epidemic diseases, but homeopathy and HP are the focus of strong debate and more research is required.<sup>26,29</sup> In accordance with the basis of HP, leptospira bacterium in highly potentised formulations might be an effective and accessible prevention alternative for the control of Leptospirosis epidemics.<sup>28,29,34,35</sup> This approach combines knowledge from homeopathy, immunology and epidemiology resulting in a possible alternative in epidemic settings.

Natural disasters cause drastic modifications of the habitat of animal vectors that increase the probability of direct contact, and of contamination of water reservoirs supplies, thus generating a sudden increase of the risk of infection of animal-borne and water-transmissible diseases.<sup>36</sup> When Leptospirosis endemic areas are affected by climatic events

producing heavy rainfall and flooding, the risk of Leptospirosis infection is dramatically boosted and challenges all prevention options.<sup>7,37–41</sup> After natural disasters in endemic regions, urgent measures are needed to control and prevent Leptospirosis epidemics but these should be based on rational and feasible strategies that integrate all available options.<sup>38,39,41</sup>

From 2005 to 2007, environmental, socio-economic and climatic changes in Cuba caused modifications of infection risks that resulted in an increase in the disease incidence. Particularly, since the beginning 2007, Leptospirosis incidence of epidemic levels was observed in a region comprising three adjacent provinces of Cuba. In October–November 2007, these three provinces were severely affected by high intensity meteorological events which caused widespread flooding, further increasing the risk of infection for the population. To confront this emergency situation, from November 2007 an intervention based on the principles of HP was carried out on this region by using a Leptospirosis nosode, based on the hypothesis that massive application of this homeopathic product would have an impact on disease incidence. This article reports the results of disease surveillance before and after this HP intervention.

## Material and methods

### Population

The entire population over 1 year of age from the provinces of Las Tunas (LT), Holguín (HG) and Granma (GR) in eastern region of Cuba, independent of their physical, psychological or social status was considered as risk group and target population. These three provinces were considered as one single geographical area, designated the Intervention Region (IR). The total population at the beginning of the study was 2,404,787 persons (LT: 534,018 HG: 1,035,388 and GR: 835,381). All the remaining provinces of Cuba were considered as another geographical area (designated Rest of the Country, RC): a total of 8,834,547 persons. The analysis involving these populations constitutes a large-scale epidemiological cohort study.

### Epidemiological surveillance

The history of Leptospirosis incidence in Cuba is recorded by an efficient National Surveillance Program (NSP) for zoonotic diseases of the Ministry of Public Health of Cuba (MPHC) established in 1980. The NSP is based on Municipal and Provincial Centres for Hygiene and Epidemiology (PCHE), connecting all Health Assistance Institutions into a national network. Regional PCHE centres have their own laboratory facilities for diagnosis and confirmation of Leptospirosis patients. After detection of suspicious cases at emergency services of Local and Provincial Hospitals, Polyclinics and Family Doctor Clinics, patient data are recorded and blood samples are submitted to PCHE for differential diagnosis. Each PCHE generates a weekly report including; suspicious cases, confirmed cases, mortality, infection risk, exposure factors and geographical–demographical distribution of

cases. A national weekly report based on provincial data is generated by the Trend Analysis Unit from the Vice-Minister of Epidemiology of the MPHC. For this paper we used the data generated by the NSP.

### Laboratory diagnosis of Leptospirosis

Leptospirosis diagnosis was assessed following the national protocol used by all diagnostic laboratories of the NSP, based on antibody detection in serum samples by HIA and haemoculture. Leptospirosis antigens for HIA were produced at the Finlay Institute, Havana, Cuba. For haemocultures, blood samples were cultured in vials containing EMHJ culture medium. Vials were incubated for several weeks at 28–30°C and checked weekly for the presence of spirochetes using dark field microscopy. Confirmed cases were reported according to the first day of symptoms. Differential diagnosis to exclude viral diseases with overlapping symptoms like hepatitis A and B and Dengue infection was performed by analysis of serum antibodies using standard ELISA methods.

### Prevention and control strategies

*Conventional measurements:* Conventional prevention strategies are based mainly on vaccination and chemoprophylaxis. The individuals treated with either vaccination or chemoprophylaxis in the IR amounted to about 3% of the population. Individuals within risk groups were vaccinated when identified with two intramuscular doses (6–8 weeks apart) of vaxSpiral<sup>®</sup> following manufacture's instructions (Finlay Institute, Havana, Cuba). This vaccine comprises three pathogenic strains (*L. interrogans* Serovar *Canicola*, *L. interrogans* Serovar *Copenhageni* and *L. kirschneri* Serovar *Mozdok*) which circulate in Cuba and Latin America. Chemoprophylaxis was applied mainly for focal treatment and outbreak control to high-risk groups when identified and consisted of a weekly oral dose of Doxycycline 100 mg.

*Homeoprophylactic strategies:* HP intervention was implemented for the entire population, over 1 year of age, of the IR. It consisted in the application of the homeopathic product nosoLEP in two different potencies. HP started in week 45 of 2007 with two oral doses of nosoLEP 200C with an interval between doses of 7–9 days. Ten to twelve months later, the schedule was completed by the administration of another two oral doses (7–9 days apart) of nosoLEP 10MC. Each dose consisted of five drops (250–300 µL) administered sublingually 20 m away from eating, smoking or drinking. It was administered by about 5000 personnel of public health system of Cuba which included family doctors, nurses, social workers and paramedics that were trained in the administration procedure. The intervention was organized and stratified in order to achieve the highest coverage in the shortest time as possible.

### nosoLEP preparation

nosoLEP is a registered product (Registration numbers: nosoLEP 200C: N-09-184-S01, nosoLEP 10MC: N-09-182-S01) developed and produced at Finlay Institute

following Good Manufacturing Practice and National Regulations for homeopathic products. nosoLEP comprises four highly-diluted strains of inactivated leptospiras: *L. interrogans* Serovar *Canicola*, *L. interrogans* Serovar *Copenhageni*, *L. kirschneri* Serovar *Mozdok* and *L. borgpetersenii* Serovar *Ballum*. The strains were selected on basis of the frequency of isolation (circulation rate), viability and virulence. Inactivated bacteria ( $10^6$  bacteria/ml) were used as source material for mother tinctures obtaining.

From the mother tinctures, 1/100 serial dilutions were prepared using homeopathic pharmaco-technical methods (Korvsakovian dilutions). Between each dilution step, the solution was succussed 100 times using an automatic dynamizer up to 200°C ( $200 \times 1:100$  dilutions) and 10 MC ( $10^4 \times 1:100$  dilutions). The four strains were processed independently and mixed in equal proportions in the final products (nosoLEP 200C and nosoLEP 10MC), in 30% ethanol. The quality of final products was controlled by measuring the alcohol content, water quality, pH and microbiologic load.

**Strains preparation:** Leptospirosis strains isolated from patients were classified at Finlay Institute using monoclonal and polyclonal reference antibodies. Virulence was checked in challenge experiments on Golden Sirius hamsters (CENPALAB, Havana, Cuba). Viable and virulent strains were cultured in EMHJ liquid media at 28–30°C until stationary phase. The cultures were harvested by centrifugation, inactivated at 56°C for 30 min and adjusted to cellular concentration of  $10^6$  bacteria/ml. Adjusted preparations were analysed for identity and inactivity by the quality control procedures established at Finlay Institute.

### Data collection

Weekly reports on Leptospirosis incidence were collected from PCHes and the Trend Analysis Unit, parts of the NSP of the MPHc. Data on rainfall were obtained from the National Institute of Hydraulic Resources (<http://www.hidro.cu/>). Population data were provided by the National Statistic Office of Cuba (<http://www.one.cu/>).

### Ethical considerations

nosoLEP is a registered product and its application is fully regulated by National Regulatory Agency according to International and National regulations for homeopathic products; it is also monitored by the National Centre for Pharmacology Surveillance. The intervention consisted in the very large-scale application of nosoLEP to the population of IR as a response to an emergency needs to confer protection to a large population exposed to an increased risk of Leptospirosis infection. We complied with international ethic standards for interventions in humans.

The massive application of nosoLEP was approved by the National Regulatory Agency and both National and Provincial Public Health Authorities. Information about the product and the intervention was provided by local TV, radio programs, newspapers and was also free available through information desks spread over the IR. Every partic-

ipant was verbally informed by the person in charge of the application and consent from each individual was obtained before administration. Consent from non-competent persons was obtained from next of kin or the person in charge. Inclusion was absolutely voluntary and free. No attempt was made to influence individuals refusing to be included.

### Statistical analysis

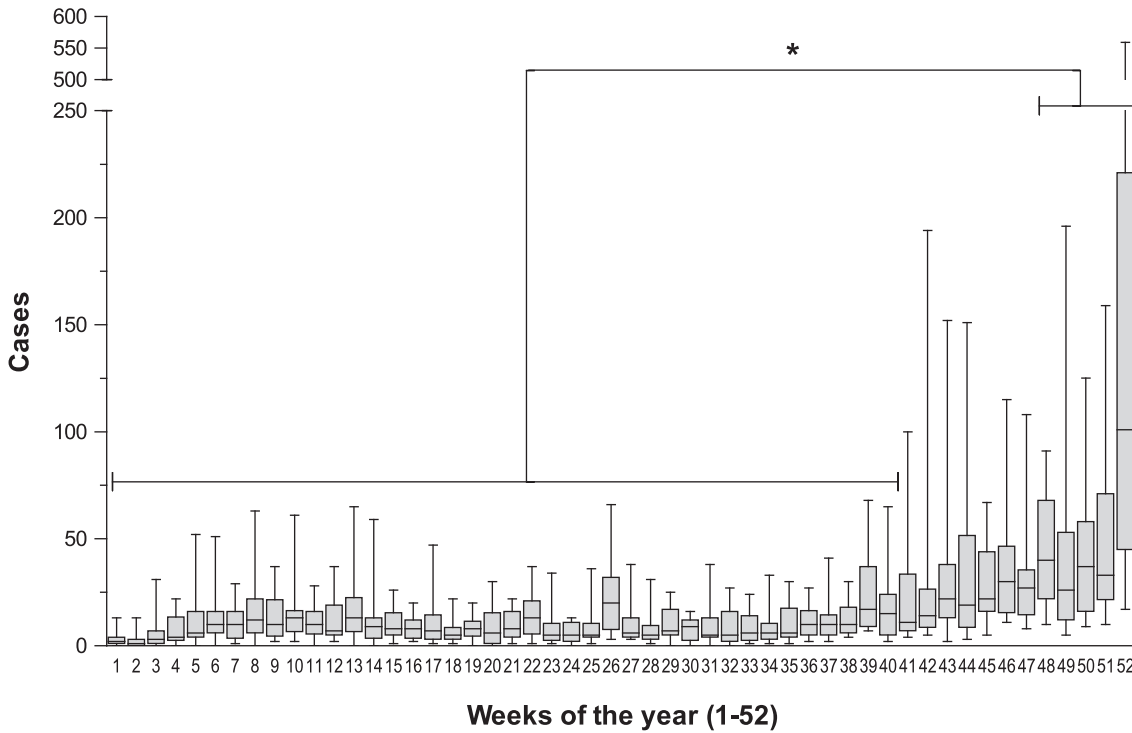
The data were analysed by combining tools from: StatGraphics Plus (Version 5.0), GraphPad Prims 4 for Windows (Version 4.00) and SPSS for Windows (Version 15.0.1). Central tendency and dispersion of weekly reports data were assessed using the median, inter-quartile range and range of data and represented by Box and Whiskers plots to explore the historic course of Leptospirosis disease. Normality of the data was assessed by Kolmogorov–Smirnov test. Differences between medians were determined by Wilcoxon signed rank test and Kruskal–Wallis test for grouped data. The Spearman correlation test was performed between cumulative rainfall and Leptospirosis cases. The Chi-squared ( $\chi^2$ ) test was used to compare the frequency of Leptospirosis infection expressed in cases  $\times 10^5$  inhabitants. Statistical significance was considered to be a 95% confidence level.

**Forecast models:** Although no model can predict exactly the future incidence of Leptospirosis, adjusted models are useful to forecast probable incidence trends and epidemics.<sup>42</sup> Five available forecast models were tested for best fit to temporal series of Leptospirosis cases (dependent) and rainfall (independent variable). To select the best fitting model, all were tested to determine how well they predicted the real temporal series of 2000–2004. The differences between forecast and real values (residual error) were analysed for statistical significance. All models gave similar forecast curves, but simple exponential smoothing was selected as no significant differences were observed when the residual errors were analysed in five out of five different tests with a 95% confidence level while the other five models all failed in one or more tests. Adjusted forecast curves, lower and upper confidence limits were validated with real data sets from different years.

## Results

### Trends of Leptospirosis incidence in Cuba

In order to better understand the behaviour of Leptospirosis infection in Cuba, the data of reported cases from 1990 to 2006 were analysed in a weekly temporal series to study the historic trends across the year. Despite the data year by year being very variable, three main periods showing a common yearly trend could be identified. The first period from weeks 1 to 40 showed a low and stable incidence with median number of reported cases remained  $<13$ /week, no significant differences were observed between weeks except week 26 (Figure 1). The data from this period also showed low variability (short inter-quartile ranges). The second period, from weeks 41 to 48 showed a slowly rising trend in the median of reported cases. In the last period (weeks 49–52), even though the dispersion



**Figure 1** Leptospirosis historic course across the year in Cuba by week (1990–2006). Box and Whisker plot of the number of cases reported by week from 1990 to 2006 in Cuba. Filled boxes showed the inter-quartile range from 75 percentile (top) to 25 percentile (bottom). Median is represented by horizontal lines in the filled boxes. Error bars showed the range of the data (maximum and minimum). \* represented significant differences between the median  $p < 0.05$  (Kruskal–Wallis test).

of the data was higher, the median values were significant higher than the medians of previous weeks and a rapid increase was observed towards the last week of the year (Figure 1).

This analysis showed that from 1990 to 2006 the historic tendency was for a higher number of confirmed cases in the last two periods of the year (weeks 41–52) with the highest infection rate in weeks 49–52. Up to 52.4% of the annual cases were reported in the last 12 of 52 weeks, and of those 53.1% were reported in the last 4 weeks of the year (weeks 49–52). The last two periods comprises from October to December, when rainfall is at its highest levels of the year.

#### Leptospirosis incidence and rains

Given the apparent relationship of Leptospirosis incidence and rains, a further correlation analysis was performed between these two variables. Unfortunately, no data on rainfall are available before 2004. However, correlation analysis from 2004 to week 46 of 2007, showed that the number of confirmed cases was significantly related to the increased rainfall (Spearman correlation factor 0.69,  $p < 0.05$ ). This suggests that the variability within the data should not be attributed to random effects and that rainfall should be considered an important risk factor for Leptospirosis infection, especially in high-risk regions.

#### The emergency

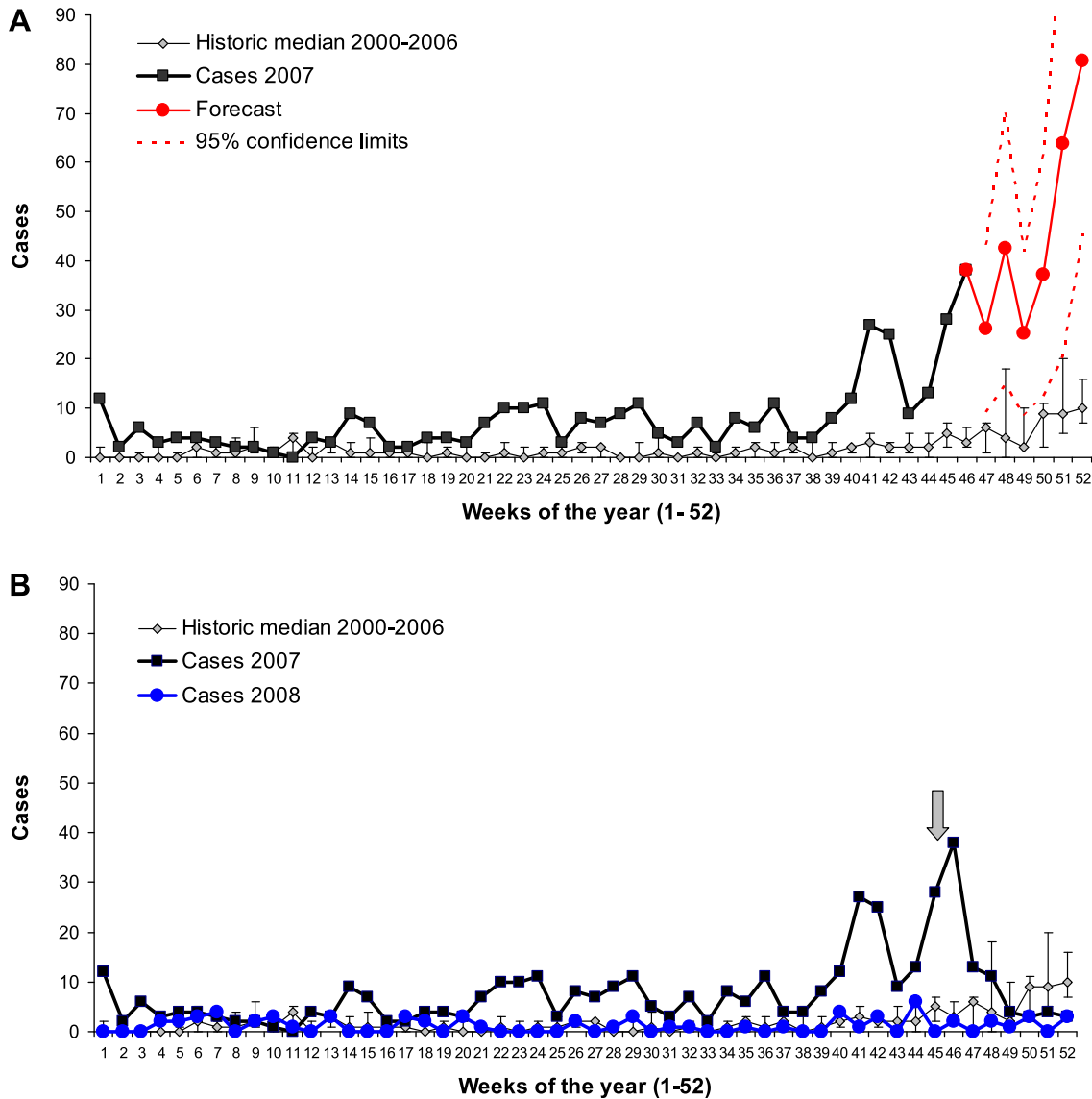
From 2005 to 2006, 43.5% of the cases of Leptospirosis reported in Cuba were concentrated in the three provinces of the IR which comprise only 21.4% of the total population of the country. The incidence trend during the year

in IR is similar to those observed historically in Cuba with the largest number of cases being reported in weeks 49–52 of the year (Figure 2A). In 2007, the incidence of Leptospirosis in the IR was higher than the historic median from the beginning and throughout the year. However, an abrupt increase was observed in the IR from weeks 39 to 46 when the number of cases increased to more than 19/week (Figure 2A).

The situation was further exacerbated by two meteorological events in October–November causing extreme rainfalls (peaks of 400 mm/h) and extensive flooding. Consequently the risks of Leptospirosis infection dramatically increased and extended the risk to the whole population.

A simple exponential smoothing model based on the 2004–2007 data was used to estimate the probable trend of the number of cases in weeks 47–52 of 2007. According to the model, no significant reduction in the confirmed cases could be expected during weeks 47–50 but a further increase in weeks 51 and 52 was forecast (Figure 2A). The forecast curves showed a trend similar to the historic observations but at higher levels than previously observed: the 95% confidence interval was 111–461 cases expected for this period.

In contrast, in the RC no significant differences were recorded between the number of cases reported in 2007 and the historic trend. In addition, the RC was not affected by any natural disasters in 2007; normal levels of rains were recorded and the model predicted a normal course of the disease (historic median and the inter-quartile ranges were included within the confidence limits of prognostic curve) for the end of the year (Figure 3A). Therefore the



**Figure 2** Leptospirosis incidence and trends in the IR. The historic course of Leptospirosis disease in the IR from 2000 to 2006 is shown for comparison in both graphics by means of the median (grey filled diamonds) of weekly confirmed cases. Errors bars represented the inter-quartile range of the median. **A.** The situation of disease at IR prior to the intervention and the predicted trend from forecast model. The black filled squares represent the number of cases reported from week 1 to 45 of 2007. After week 45, the red filled circles represent the forecast trend predicted by simple exponential smoothing model adjusted to the data of the region (2000–week 45 of 2007). Dotted lines represent the 95% confidence limits for the forecast curve. **B.** Shows the follow up of confirmed cases after the start of large-scale application of HP leptospira nosode (nosoLEP) in the IR from week 45 of 2007. The black filled squares represent the number of cases reported before and after the start of the intervention in 2007. The start of the intervention in 2007 is denoted by the vertical arrow. The number of cases reported in the following weeks of the whole 2008 year is represented by blue filled circles. At week 48 2007 the coverage was over 70% and 92% at week 50. Significant differences ( $p < 0.05$ ) were detected at weeks 50–52 of both 2007 and 2008 when compared with the corresponding historic medians (Wilcoxon signed rank test).

probability of occurrence of a major epidemic was extremely high in the IR while normal historic behaviour of the disease was expected in the RC.

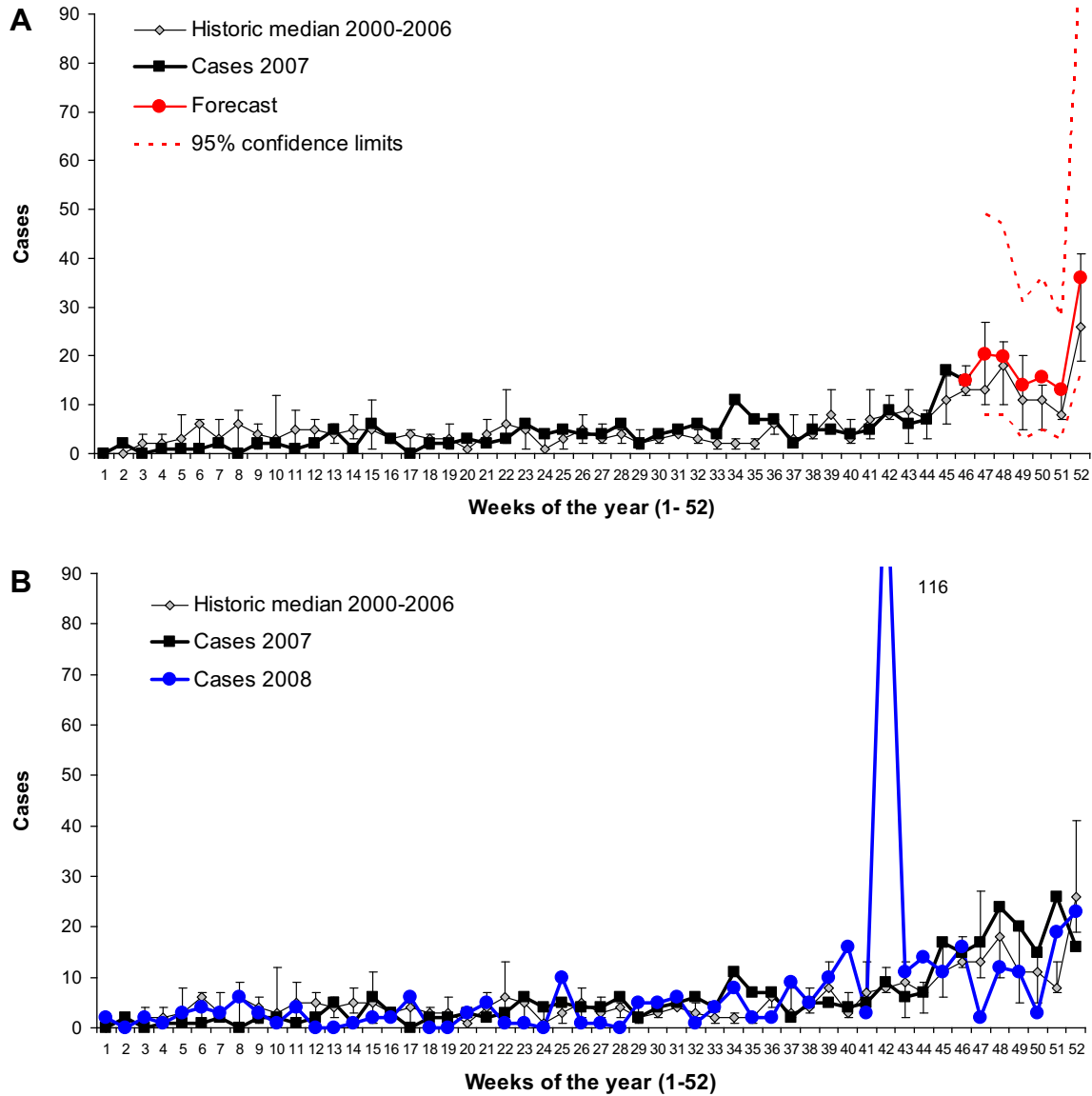
**The intervention in 2007**

Considering the epidemic situation in the IR, the unfavourable prognosis and the emergency caused by natural disasters, a massive HP application of nosoLEP 200C was started at week 45, 2007. vaxSpiral<sup>®</sup> vaccination and chemoprophylaxis in high-risk groups were continued but because of the limited availability of vaccines, vaccination

coverage among newly exposed population was limited to 15,000 individuals in the IR (0.6% coverage). In contrast, HP coverage reached over the 92% of total population of IR at week 50 representing 2,112,257 individuals treated with two doses of nosoLEP 200C.

**Surveillance results from 2007 (end of the year)**

The impact of the intervention was followed through the surveillance system of MPHIC. Two weeks after the intervention started, a dramatic decrease in the number of confirmed cases was observed in the IR, falling from 38 cases in week 46 to 3–4 cases/week during weeks



**Figure 3** Leptospirosis incidence and trends at the RC (no intervened region, RC). The historic course of Leptospirosis disease in the RC from 2000 to 2006 is shown for comparison median (grey filled diamonds) of weekly confirmed cases. Errors bars represented the inter-quartile range of the median. **A.** The RC from week 1 to week 45 of 2007 (the time frame before the intervention in the IR) is shown in black filled squares. After week 45, the probable trend of cases predicted by simple exponential smoothing model adjusted to the data of the region (2000–week 45 of 2007) is plotted in red filled circles. Discontinuous lines represent the 95% confidence limits for the forecast curve. **B.** The weekly confirmed cases during 2007 in the RC are represented by black filled squares while the number of cases of 2008 is represented by filled blue circles. Only conventional measurements and no HP approaches were applied in this region (RC). No significant differences were detected between the number of reported cases on weeks 49–59 of both 2007 and 2008 and the corresponding historic medians (Wilcoxon signed rank test).

49–52. The number of cases detected in weeks 49–52 of 2007 was significantly lower than the historic median for these weeks (Figure 2B). A noteworthy finding was that this reduction in the Leptospirosis cases occurred in only 3 weeks and was coincident with the achievement of a 70% coverage of the population treated with nosoLEP 200C (Figure 2B). When comparing with the predicted trend, the total confirmed cases after intervention (weeks 47–52) was reduced from a forecast 111–461 (95% confidence limits) to 38 representing a reduction of 91.8–65.8% (Figure 2B).

Similar analysis was done for the RC to determine whether a similar phenomenon was observed in the un-

treated regions. In the same time period (weeks 47–52, 2007) the numbers of confirmed cases in RC were not statistically different from the historic medians. In agreement with the prediction, the number of confirmed cases in RC remained over 16 cases/week at the end of 2007 (Figure 3B).

#### Surveillance results from 2008

The incidence of Leptospirosis was also followed in 2008 to examine the incidence over a full year. Two outstanding factors should be considered for the analysis in 2008. The first is the impact of three high intensity hurricanes that affected almost all the country in August–September and generated very heavy rain (Hurricanes

**Table 1** Summary of data in IR, RC and the whole country. Data for the total population, treated population (homeoprophylactic intervention), confirmed Leptospirosis cases and incidence of the disease ( $10^5$  inhabitants) on 2007 and 2008 are showed

Region	Population		Treated		Confirmed cases**			Incidence per $10^5$ inhabitants	
	$10^6$ people	%	$10^6$ people	%	2007	2008	% of 2007	2007	2008
IR	2405	21.4	2309	96	401	64	16.0	16.7	2.7
RC	8834	78.6	0	0	309	376	121.7	3.5	4.3
Cuba	11,239	100	2309	20.5	710	440	62.0	6.3	3.9

\*\*Leptospirosis infected cases confirmed by laboratory tests.

‘Gustav’, ‘Ike’ and ‘Paloma’). The second factor was the completion of the application of nosoLEP 10MC (reinforcement treatment) in 96% population of IR in September 2008 (2,308,562 people).

From weeks 1 to 41 of 2008, both RC and IR reported a number of confirmed cases similar to the historic median (Figure 2B and 3B). In week 42 an outbreak of the disease was reported in a closed population of RC but fortunately was quickly controlled by chemoprophylaxis. But at the end of the year major differences in the number of cases between RC and IR were detected. In the weeks 49–52 of 2008 the number of cases in IR remained significantly lower than the historic median, thus the modification on the trends observed in 2007 persisted a year after the initiation of the HP intervention (Figure 2B). Additionally, in the IR in 2008, in 24 out of 52 weeks there were no confirmed cases and in 40 of 52 weeks, 0–2 cases/week (Figure 2B). In contrast, the number of cases in the RC remained similar to historic levels with a high number of infected people at the last weeks of the year and no change in the trend in either 2007 or 2008 (Figure 3B). Despite the increased risks of Leptospirosis infection in IR and four meteorological disasters, the annual number of cases was significantly decreased from 401 in 2007 to 64 cases/year in 2008, a reduction of 84% (Table 1). However in the untreated RC region, 67 more cases were reported in 2008 than in 2007, an increase of 21.7%. Due to the decrease

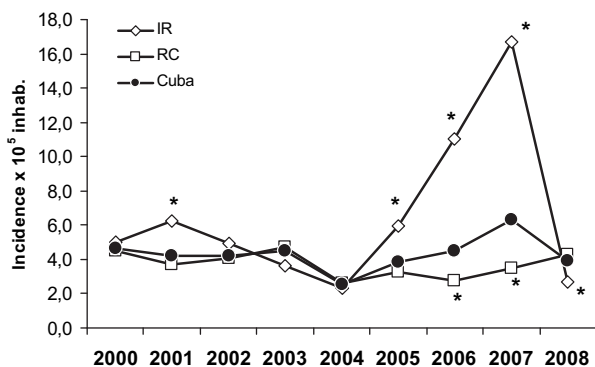
in the IR there was an overall reduction of 62% in the annual cases confirmed in Cuba (Table 1).

Additional evidence was obtained from the analysis of the incidence of the disease (per  $10^5$  inhabitants) by year. During the period 2000–2004, the incidence of the disease in RC and IR was not statistically different, with the exception of 2001 (Figure 4).

In contrast, from 2005 to 2007, the incidence of Leptospirosis in IR increased to reach its highest value in 2007 of 16.6 per  $10^5$  inhabitants. In 2008, the incidence in the IR decreased to 2.7 per  $10^5$  inhabitants and was significantly lower than RC and Cuba. Consequently, the reduction observed in Cuba from 6.3 (2007) to 3.9 (2008) was the result of the decrease in the IR (Figure 4).

The differences in the IR and RC regarding Leptospirosis incidence were also evident when correlating the disease with the rainfall. From 2004 and up to 2007, Leptospirosis incidence by year was closely related to rainfall in both regions (Figure 5A and 5B). However, in 2008 this correlation was not observed in IR since the decrease in the incidence was not proportional to rainfall (Figure 5A). In contrast, in RC no significant difference was observed in the correlation between rainfall and Leptospirosis cases in 2008 (Figure 5B).

Summarizing the data from week 46, 2007 to week 52, 2008, major differences between RC and IR were demonstrated. Both the trends and the incidence of Leptospirosis were significantly modified in IR with a reduction of confirmed cases, while remaining as expected in the RC.

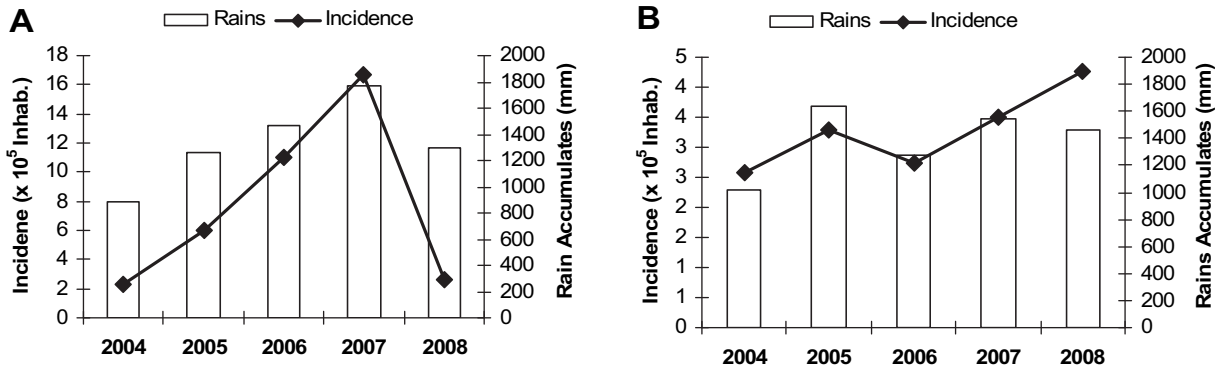


**Figure 4** Incidence of Leptospirosis (per  $10^5$  inhabitants) from 2000 to 2008 in Cuba. The country was divided in two regions: IR: where at the end of 2007 a homeoprophylactic formulation against Leptospirosis (nosoLEP) was applied to 96% ( $2.3 \times 10^6$  people) of total population of the region. RC: no other intervention than established measurements for Leptospirosis control was applied. The total national incidence (filled circles) is also represented. \* denotes significant differences compared to the incidence of Cuba by Chi-square ( $\chi^2$ ) test ( $p < 0.05$ ).

## Discussion

As with most zoonotic diseases, the risks of Leptospirosis infection are multifactorial and comprise environmental, economical, social, and host factors. Therefore, control measures including education, vector control, sanitation and protective immunization should be well integrated and locally adapted in strategies to deliver multipoint reduction of the main risk factors. In endemic regions the strategies must be applied extensively and continuously to achieve a significant impact on the incidence.<sup>43</sup> Nevertheless given the strong influence of environmental factors, control of the disease is difficult and epidemic outbreaks may be frequent and sometimes unavoidable in endemic areas. A more effective impact on disease incidence can be achieved by preventive alternatives like vaccination although the effectiveness of such interventions depends on the appropriate identification of the





**Figure 5** Relation of rainfall and incidence of Leptospirosis from 2004 to 2008. Bars represent the total rainfall (right axis) by year and lines showed the incidence of the disease expressed as confirmed cases per 10<sup>5</sup> inhabitants (left axis). **A.** IR: where a homeoprophylactic Leptospirosis nosode (nosoLEP) was applied to 96% (2.3 × 10<sup>6</sup> people) of total population of the region at the end of 2007. **B.** RC: receiving only the standard measures for Leptospirosis control. No homeoprophylactic approach was applied in this region.

population at risk, the size of risk groups, the availability of vaccines and the coverage of vaccine application.<sup>18</sup>

Leptospirosis morbidity in Cuba from 1990–2006 showed a trend of increasing number of confirmed cases towards the end of every year (last 8 weeks), particularly weeks 49–52. The causes underlying this observation are multiple but the rainfall appears to be a strong influencing factor since a high positive correlation with number of reported cases was observed. Thus, control measurements should be strengthened at the end of the year and special attention should be given to unexpected increases in rainfall.

From 2004 and up to 2007 an increase in the annual incidence of the disease was observed in Cuba, particularly in the IR although there had been no modifications in the strategies for Leptospirosis control. The main cause of this observation is likely to be the implementation of policies promoting agriculture and animal breeding that caused rapid and continuous changes in the size and composition of risk groups, making identification difficult. The confirmed cases reported from 2004 were mainly due in individuals over 15 years of age suggesting that the continuous modification of risk groups affected the coverage of vaccination and resulted in an increased number of unprotected people exposed to the infection.

The incidence of Leptospirosis reached epidemic levels on 2007 in the IR when the number of cases per week was over the historic median of the region from the beginning of the year. Furthermore, an emergency epidemiologic situation developed in the IR after the impact of meteorological disasters associated with heavy rain, extensive flooding and extensive damage to the environment. The sudden and drastic increase of Leptospirosis infection risk and of the number of exposed individuals also reduced the applicability and effectiveness of conventional measurements.

A forecast model was used to predict the possible trend of Leptospirosis infection during the period of higher infection risks on 2007 in both IR and RC. Because these two regions presented different epidemic and meteorological conditions, the model showed different prediction curves. In the IR a high probability of significant increases in the number

of cases from week 46 was forecast while no changes in the historic trends of the disease were predicted for RC.

The HP intervention against Leptospirosis was implemented (starting at week 45 2007) in the IR as a response to the emergency situation with the main objective to protect about 2.3 million people exposed to an alarming epidemiological prognosis. Analysis of the data from the Leptospirosis surveillance demonstrated major differences between IR and RC regarding disease incidence and trends after the intervention. Follow up in the IR showed that the historic trend of the disease was drastically modified from the end of 2007 and throughout 2008 since the number of confirmed cases was significantly reduced. Unexpectedly this reduction was evident only 2 weeks after the start of HP intervention and was sustained up to last weeks of year 2008. A noteworthy finding was that in both 2007 and 2008, the number of cases at IR remained below the historic median during the period of the year of the highest risk of infection (weeks 49–52). No modifications in Leptospirosis morbidity were recorded in the RC but there was an increase of reported cases in 2008. Particularly at the end of 2007, the real number of cases in the RC showed a trend similar to that predicted by the forecast model suggesting the validity of the model to estimate the prevalence of the disease based on the historic records and rainfall.

Analysing the annual incidence in both regions, IR showed a reduction of 84% while RC reported an increase of 21.7% in the number of Leptospirosis cases from 2007 to 2008; the incidence of the disease in IR dropped from 16.7 × 10<sup>5</sup> (2007) to 2.7 × 10<sup>5</sup> (2008). This reduction in IR was achieved even when the risk for infection remained at a high level. Finally, the correlation between rainfall and incidence was disrupted in IR in 2008 but sustained in the RC. The effect observed after the HP intervention in the IR had an impact on the disease incidence of the whole of Cuba which dropped to 3.9 × 10<sup>5</sup> inhabitants in 2008.

According to the data, the modification of Leptospirosis incidence observed in IR could be considered as unique phenomena of this region that cannot be explained either by historic trends of the disease or by changes in the rainfall. Considering that there are multiple possible causes of the differences detected between IR and RC, the analysis

should be focussed on the relative effect of the following factors on Leptospirosis incidence:

First, the risks for Leptospirosis infection are present all across the country.

Second, both high circulation of the pathogen (epidemic levels) and environmental conditions (four natural disasters) increased the risk of infection at IR to a greater extent than in RC. During 2007 the RC was affected neither by strong rainfalls nor by high epidemic rates of Leptospirosis infection. However in 2008, both regions were affected by hurricanes and similar levels of rains were recorded but no increase in Leptospirosis incidence was observed in IR.

Third, the coverage of conventional measurement of control including vaccination and chemoprophylaxis was similar in both regions since their application followed the current guidelines from MPHIC. The main difference regarding prevention measures was the large-scale HP intervention in the IR.

Fourth, the extent of vaccination and HP was very different in the IR. The HP intervention covered over 96% of the target population while the coverage of vaccination was limited to 0.6% because of the reduced stockpile of the vaccine vaxSpiral<sup>®</sup> at that time.

Fifth, the reduction in the number of confirmed cases in IR occurred within 2 weeks but was sustained for the next 57 weeks. This sharp decrease of incidence does not suggest an expected effect of vaccination or chemoprophylaxis considering the time needed to induce a protective immune response by vaccines and the short temporal protection of antibiotics. In fact, because of the vaccination schedule of vaxSpiral<sup>®</sup>, the immunization of newly exposed individuals was finished in a time frame several weeks after the effects observed at IR. The reduction of confirmed cases on IR was coincident with the achievement of 70% of coverage of HP treatment.

Taken together, these facts suggest that the HP intervention in IR was the main factor causing a significant reduction in Leptospirosis incidence. However, the effectiveness of homeopathy has been widely discussed and remains controversial despite decades of research and clinical testing. Double blind controlled and randomised clinical trials have been seen as the gold standard to demonstrate efficacy of any health intervention. One limitation of such trials is often the size of the population and the levels of exposure to risk factors. Thus, even when successful results are obtained from controlled trials demonstrating efficacy, the real effectiveness needs to be tested in large populations with high exposure to the target disease, preferably in endemic areas.

Taking into account that the HP intervention was implemented in a large population of a high-risk endemic area, the data strongly suggest high effectiveness of HP and support its applicability to control of epidemic disease.

However, there is no evidence supporting the replacement of conventional strategies and no data regarding efficacy are presented. The rational design of combined strategies to confront a complex epidemic situation should improve the effectiveness of control measurements. HP might synergistically complement prevention strategies

to reduce the incidence of diseases in the short term by inducing protective status in the target population. Nonetheless, other issues including economic, accessibility, availability, adverse reactions and timing should be considered for the appropriate design of prevention strategies. The massive application of HP in the IR also showed potential regarding feasibility and time saving. When trained and organized personnel are involved in the HP application, large coverage can be achieved in a short time with modest resources.

Further studies in the IR should give new evidence to support stronger conclusions. The comparative study of mechanisms of immunity within the HP treated, vaccinated or not vaccinated population could provide important data to complement the epidemiological observations. Nonetheless, the surveillance of the Leptospirosis incidence in Cuba over the next years should provide valuable evidence of the effect of HP intervention in IR.

## Conclusions

The homeoprophylactic intervention was strongly associated with a drastic reduction of disease incidence resulting in complete control of the epidemic. The results support the use of homeopathic prophylactic formulations as a feasible strategy to help control epidemic situations. Integrated approaches should be designed according to regional conditions and epidemic characteristics. Scientific rigour and responsibility should direct further research and application of HP.

## Competing interest

The authors declare that they have no competing interests.

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## References

- 1 Pappas G, Papadimitriou P, Siozopoulou V, Christou L, Akritidis N. The globalization of leptospirosis: worldwide incidence trends. *Int J Infect Dis* 2008; **12**(4): 351–357.
- 2 Vijayachari P, Sugunan AP, Shriram AN. Leptospirosis: an emerging global public health problem. *J Biosci* 2008; **33**(4): 557–569.
- 3 Cruz LS, Vargas R, Lopes AA. Leptospirosis: a worldwide resurgent zoonosis and important cause of acute renal failure and death in developing nations. *Ethn Dis* 2009; **19**(1 Suppl 1): S1–S37–S41.
- 4 Margaletic J. Small rodents in the forest ecosystem as infectious disease reservoirs. *Acta Med Croatica* 2003; **57**(5): 421–426.
- 5 Adler B, de la Pena Moctezuma A. Leptospira and leptospirosis. *Vet Microbiol* 2010; **140**(3–4): 287–296.
- 6 Levett PN. Leptospirosis. *Clin Microbiol Rev* 2001; **14**(2): 296–326.

- 7 Guerra MA. Leptospirosis. *J Am Vet Med Assoc* 2009; **234**(4): 472–478, 430.
- 8 Nachega JB, Bottieau E, Zech F, et al. Travel-acquired scrub typhus: emphasis on the differential diagnosis, treatment, and prevention strategies. *J Travel Med* 2007; **14**(5): 352–355.
- 9 Sehgal SC. Epidemiological patterns of leptospirosis. *Indian J Med Microbiol* 2006; **24**(4): 310–311.
- 10 Monahan AM, Miller IS, Nally JE. Leptospirosis: risks during recreational activities. *J Appl Microbiol* 2009; **107**(3): 707–716.
- 11 Reis RB, Ribeiro GS, Felzemburgh RD, et al. Impact of environment and social gradient on *Leptospira* infection in urban slums. *PLoS Negl Trop Dis* 2008; **2**(4): e228.
- 12 Palaniappan RU, Ramanujam S, Chang YF. Leptospirosis: pathogenesis, immunity, and diagnosis. *Curr Opin Infect Dis* 2007; **20**(3): 284–292.
- 13 Kobayashi Y. Human leptospirosis: management and prognosis. *J Postgrad Med* 2005; **51**(3): 201–204.
- 14 Kobayashi Y. Clinical observation and treatment of leptospirosis. *J Infect Chemother* 2001; **7**(2): 59–68.
- 15 Stoddard RA, Gee JE, Wilkins PP, et al. Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. *Diagn Microbiol Infect Dis* 2009; **64**(3): 247–255.
- 16 Brett-Major DM, Lipnick RJ. Antibiotic prophylaxis for leptospirosis. *Cochrane Database Syst Rev* 2009;(3): CD007342.
- 17 Guidugli F, Castro AA, Atallah AN. Antibiotics for preventing leptospirosis. *Cochrane Database Syst Rev* 2000;4. CD001305.
- 18 Fitzgerald J. Availability of leptospirosis vaccine. *Vet Rec* 2009; **164**(5): 157.
- 19 Martinez Sanchez R, Obregon Fuentes AM, Perez Sierra A, et al. The reactogenicity and immunogenicity of the first Cuban vaccine against human leptospirosis. *Rev Cubana Med Trop* 1998; **50**(2): 159–166.
- 20 Martinez R, Perez A, Quinones Mdel C, et al. Efficacy and safety of a vaccine against human leptospirosis in Cuba. *Rev Panam Salud Publica* 2004; **15**(4): 249–255.
- 21 Martinez Sanchez R, Perez Sierra A, Baro Suarez M, et al. Evaluation of the effectiveness of a new vaccine against human leptospirosis in groups at risk. *Rev Panam Salud Publica* 2000; **8**(6): 385–392.
- 22 Sanchez RM, Sierra AP, Obregon Fuentes AM, et al. Reactogenicity and immunogenicity of Cuban trivalent inactivated vaccine against human leptospirosis in different vaccination schedules. *Rev Cubana Med Trop* 2002; **54**(1): 37–43.
- 23 Obregon AM, Martinez G, Martinez R, et al. Serological response by ELISA and MAT in Cuban volunteers vaccinated with vax SPIRAL. *Rev Cubana Med Trop* 2004; **56**(2): 148–151.
- 24 Rodriguez I, Martinez R, Zamora Y, et al. Response of antileptospira IgG antibodies in individuals immunized with vax-SPIRAL. *Rev Cubana Med Trop* 2005; **57**(1): 32–37.
- 25 Wang Z, Jin L, Wegrzyn A. Leptospirosis vaccines. *Microb Cell Fact* 2007; **6**: 39.
- 26 Castro D, Nogueira GG. Use of the nosode meningococcinum as a preventative against meningitis. *J Am Inst Homeopath* 1975; **68**: 211–219.
- 27 Leary B. The homoeopathic management of cholera in the nineteenth century with special reference to the epidemic in London, 1854. *Med Gesch* 1997; **16**: 125–144.
- 28 Jonas WB. Do homeopathic nosodes protect against infection? An experimental test. *Altern Ther Health Med* 1999; **5**(5): 36–40.
- 29 Mroninski CRL, Adriano EJ, Mattos G. Meningococcinum, its protective effect against meningococcal disease. *Homeopath Links* 2001; **14**(4): 230–234.
- 30 Golden I. *Vaccination & homoeoprophylaxis? A review of risk and alternatives*. 6th edn. Canberra: Isaac Golden Publications, 2005 (2007) 1–240.
- 31 Vickers AJ, Smith C. Homeopathic oscillococinum for preventing and treating influenza and influenza-like syndromes. *Cochrane Database Syst Rev* 2006; **3**: CD001957.
- 32 Dean M. Comparative evaluation of homeopathy and allopathy within the Parisian hospital system, 1849–1851. *J R Soc Med* 2010; **103**(1): 34–36.
- 33 Pruna PM. Homeopathic immunization against yellow fever in Havana, 1855. *Asclepio* 1991; **43**(2): 59–68.
- 34 de Almeida LR, Campos MC, Herrera HM, et al. Effects of homeopathy in mice experimentally infected with *Trypanosoma cruzi*. *Homeopathy* 2008; **97**(2): 65–69.
- 35 Shah-Rossi D, Heusser P, Baumgartner S. Homeopathic treatment of *Arabidopsis thaliana* plants infected with *Pseudomonas syringae*. *Scientific WorldJournal* 2009; **9**: 320–330.
- 36 Watson JT, Gayer M, Connolly MA. Epidemics after natural disasters. *Emerg Infect Dis* 2007; **13**(1): 1–5.
- 37 Kawaguchi L, Sengkeopraseuth B, Tsuyuoka R, et al. Seroprevalence of leptospirosis and risk factor analysis in flood-prone rural areas in Lao PDR. *Am J Trop Med Hyg* 2008; **78**(6): 957–961.
- 38 Campanella N. Infectious diseases and natural disasters: the effects of Hurricane Mitch over Villanueva municipal area, Nicaragua. *Public Health Rev* 1999; **27**(4): 311–319.
- 39 Sanders EJ, Rigau-Perez JG, Smits HL, et al. Increase of leptospirosis in dengue-negative patients after a hurricane in Puerto Rico in 1996 [correction of 1966]. *Am J Trop Med Hyg* 1999; **61**(3): 399–404.
- 40 Niwetpathomwat A, Niwatayakul K, Doungchawee G. Surveillance of leptospirosis after flooding at Loei Province, Thailand by year 2002. *Southeast Asian J Trop Med Public Health* 2005; **36**(Suppl. 4): 202–205.
- 41 Sehgal SC, Sugunan AP, Vijayachari P. Outbreak of leptospirosis after the cyclone in Orissa. *Natl Med J India* 2002; **15**(1): 22–23.
- 42 Tan H, Ping W, Yang T, et al. The synthetic evaluation model for analysis of flooding hazards. *Eur J Public Health* 2007; **17**(2): 206–210.
- 43 John TJ. The prevention and control of human leptospirosis. *J Postgrad Med* 2005; **51**(3): 205–209.