Acta Tropica 170 (2017) 79–84

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

# A cross-sectional epidemiological study of domestic animals related to human leptospirosis cases in Nicaragua

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ARTICLE INFO

*Article history:*
Received 29 July 2016
Received in revised form 23 February 2017
Accepted 26 February 2017
Available online 28 February 2017

*Keywords:*
Leptospirosis
Domestic animals
Nicaragua
Human cases

ABSTRACT

Leptospirosis is one of the most extended zoonosis worldwide and humans become infected most commonly through contact with the urine of carrier animals, either directly or via contaminated water or soil. The aim in this study was to analyse the epidemiological behaviour of *Leptospira* spp., from domestic animals around the sites of human leptospirosis cases in Nicaragua, from 2007 through 2013. We report the results of a cross-sectional epidemiological study with a non-probability sampling of blood ($n = 3050$) and urine ($n = 299$) from Domestic Animals (DA) around the sites of human leptospirosis cases in Nicaragua. We analysed data obtained through Microscopic Agglutination Test (MAT), *in-vitro* culture, real time PCR and sequencing of lfb1 *locus*. Frequencies of 30.31% (95% CI: 28.66–31.95) and 15.38% (95% CI: 11.12–19.64) were obtained from serological test and from *in-vitro* culture, respectively. Although similar frequencies from serology test ($P \geq 0.05$) were found in DA species, *in-vitro* culture frequencies were significantly higher from bovine, equine and sheep ($P < 0.05$) in comparison with swine and canine species. Ten serogroups of pathogenic *Leptospira* spp. were encountered, with the highest presence of Icterohaemorrhagiae serogroup 34.65% (95% CI: 29.35–39.94). We identified 7 samples homologous to *L. interrogans* species Pyrogenes serovar and 3 samples as *L. noguchii* Louisiana or Panama serovars by analysis of lfb1 sequences. We were able to establish a temporal and spatial correlation from DA and cumulative incidence of human cases. Therefore an effective epidemiological surveillance should be implemented with a specific control program toward DA in order to reduce human leptospirosis incidence.



## 1. Introduction

Leptospirosis is a serious threat to public health and is considered the most extended zoonosis worldwide. Humans usually become infected with leptospires through direct or indirect exposure to the urine of infected wild or DA, which frequently occurs in developing countries with poor sanitation where humans and animals often live in close proximity (Reller et al., 2014; Thiermann, 1984; Wasiński and Dutkiewicz, 2013).

The *Leptospira* life cycle involves haematogenous dissemination to the kidneys, shedding in the urine, persistence in the environment and acquisition of a new host (Haake and Levett, 2015). DA with subclinical infections as well as those who recover from the clinical disease become an important source of infection for humans and other hosts, since they continue shedding leptospires for a long time (Faine, 1957; Valverde et al., 2008). It is worth noting that transient leptospire shedding does occur during human infection, but human-to-human transmission is extremely rare (Haake and Levett, 2015).

Leptospirosis is a good example of the interaction between humans and their environment, and particularly human interface with DA (Lau et al., 2010). The countries most affected are those located in tropical and subtropical areas, where conditions such as temperatures, relative humidity, rainfall, structure, soil composition and pH are optimal for the pathogen to survive and multiply (Wasiński and Dutkiewicz, 2013; Schneider et al., 2012).

Leptospirosis has acquired increased worldwide attention following epidemics characterized by severe pulmonary haemorrhage syndrome without jaundice or renal complications. This syndrome

*Abbreviations:* DA, domestic animals; MINSA from its Spanish acronym, Ministry of Health; PAHO, Pan American Health Organization; MAT, Microscopic Agglutination Test; CI, confidence intervals; $X^2$, Chi square test.

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http://dx.doi.org/10.1016/j.actatropica.2017.02.031
0001-706X/

was originally diagnosed in China and Korea, then in Nicaragua following hurricane flooding in rural areas (Lehmann et al., 2014; Park et al., 1989; Trevejo et al., 1998; Zaki and Shieh, 1996). In Nicaragua, several human leptospirosis outbreaks with end-stage pulmonary haemorrhage have been observed since 1995, most notably in 1998 and 2007 (Ashford et al., 2000; Trevejo et al., 1998). Leptospirosis behaves as an endemic disease in urban and rural areas, with average notification of 2 cases per week in the rainy season (May–November). This average might rise to 8 cases per week in the West Region (Departments of León and Chinandega), where outbreaks occur more frequently (Schneider et al., 2012).

In the present report, we analysed the epidemiological behaviour of *Leptospira* spp. from DA around the sites of human leptospirosis cases in Nicaragua from 2007 through 2013. This study constituted part of the national plan for the prevention and control of leptospirosis, supported and integrated by the MINSA, Ministry of Agriculture and Forestry (MAGFOR), National Autonomous University of Nicaragua-León (UNAN-León, from its Spanish acronym) and the PAHO.

## 2. Material and methods

### 2.1. Study setting

We conducted a cross-sectional epidemiological study from 2007 through 2013 in 16 out of 17 Departments of Nicaragua, in Central America, located between 10°45′ and 15° 15′ North latitude and 83°00′ and 88° 00′ West longitude. This region is frequently affected by natural disasters. The number of bovine reaches around 4 million heads reflecting the importance of cattle ranching in the country.

### 2.2. Sampling

A non-probability sampling was conducted upon MINSA notification of 406 human leptospirosis cases. Whole blood and urine samples were collected from DA found nearby the human patient's residence, which included bovine, canine, equine, sheep and swine species. We conducted sampling within a 30 m diameter surrounding the patient's residence in urban areas, and up to 100 m diameter in rural areas.

### 2.3. Serological testing by MAT

The MAT to detect specific leptospira's antibodies was performed using a standard microtiter method. We searched for antibodies against *L. interrogans* (*sensu lato*) using twenty-eight reference pathogenic strains along with Patoc 1 and ICF strains, which represented non-pathogenic species (Table S1).

### 2.4. Culture of Leptospira spp.

Approximately 2.5 ml of urine were filtered through a 0.45 μm nitrocellulose membrane, and 0.5 ml of the filtrate was inoculated into 3 ml of Ellinghausen-McCullough-Johnson-Harris (EMJH) liquid medium (Difco, USA) supplemented with 5-fluorouracil (200 μg/ml) and enriched with 1% rabbit serum.

The cultured tubes were incubated between 28 and 30 °C for a 3-month period and at least once a week a 10 μl droplet from each culture was examined under dark-field microscopy for lepstospires presence.

### 2.5. Pathogenic leptospire identification

To determine whether these isolates were pathogenic or non-pathogenic leptospires, spirochetes from previously obtained cultures were reactivated in the EMJH medium, and after 7 days post-incubation, the media were centrifuged at 16,000 rpm for 10 min. The supernatant was discarded and 200 μl of re-suspended precipitate was used for DNA extraction, following the manufacturer's instruction (UltraClean® bacterial® DNA Culture kit MO BIO, USA).

Molecular analyses were performed in the Molecular Diagnostic Laboratory of the Infectious Diseases and Epidemiology Unit, Faculty of Veterinary Sciences, University of Zaragoza (Spain). We performed the Real time PCR (qPCR) with primers previously described (Levett et al., 2005), LipL32 270F (CGCTGAAATGGGAGTTCGTATGATT), LipL32 692R (CCAACAGATGCAACGAAAGATCCTTT), which amplify a 423 bp fragment of LipL32 gen, that is believed to be conserved in the pathogenic serovars. Samples were run in triplicate, nuclease free water was included as a negative control and DNA from a pure culture of Pomona strain was used as a positive control.

### 2.6. Genotyping

Isolates with results under 34 CT value were analysed by sequencing of lfb1 *locus* (331 bp) (Perez and Goarant, 2010). DNA purifications were performed with the commercial kit according to the manufacturer UltraClean® 15 DNA Purification MO BIO, USA. The sequences of the *locus* of each strain were performed in the Laboratory Service Sequencing and Functional Genomics, University of Zaragoza (Spain) and were aligned using ClustalW 1.6 software. The history of evolution was inferred using the neighbor-joining method (Saitou and Nei, 1987) and the analysis were conducted using the MEGA6 software (Tamura et al., 2013).

### 2.7. Data sources and statistical analysis

An epidemiological questionnaire was filled for each DA in the study. Relative frequencies with 95% CI were calculated for statistical descriptive analyses. A $X^2$ or Fisher exact test were applied to compare between variable categories. For the control of confounding variables, stratifications and logistic regression models were applied. Cohen's kappa index was calculated to compare the results of the MAT and direct culture techniques (Agresti and Kateri, 2011). All data were recorded and analysed in statistic software Package for Social Sciences Software (SPSS, version 19).

Our results were compared with human case cumulative incidence rate (per 10,000 inhabitants), as previously reported by other authors (Sánchez, 2012; Schneider et al., 2012; Soto, 2012), and with epidemiological bulletins published on the MINSA official website (http://www.minsa.gob.ni/).

### 2.8. Ethics statement

The procedures were performed by veterinary staff of the School of Veterinary Medicine, UNAN-León. We requested in each case the acceptance and approval of the patient through an informed written consent statement.

## 3. Results

A total of 3050 whole blood and 299 urine samples were collected from DA. Sampling was conducted upon MINSA notification of 406 human leptospirosis cases from 2007 through 2013.

### 3.1. Correlation of results obtained by different techniques

Frequencies of seropositive samples by MAT and *in-vitro* culturing from DA were 30.31% (95% CI: 28.66–31.95) and 15.38% (95% CI: 11.12–19.64), respectively. A Cohen's Kappa value of 0.176